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Fig. 1A

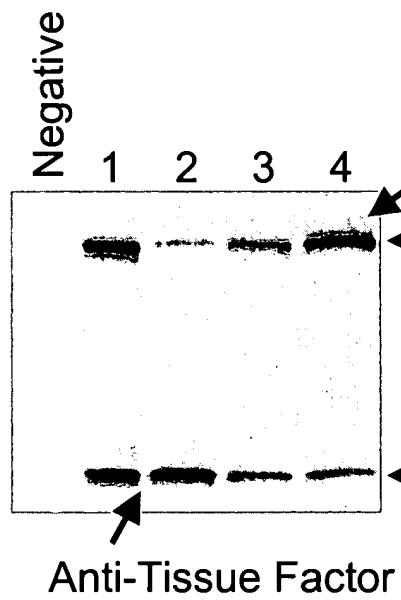


Fig. 1B

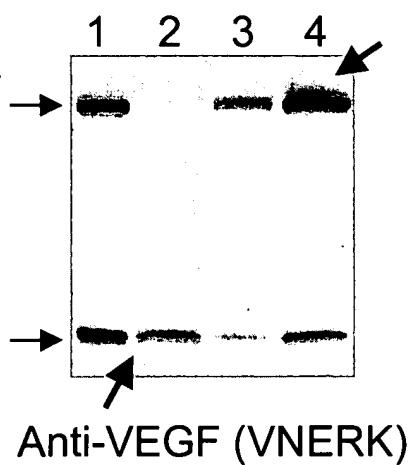


Fig. 2

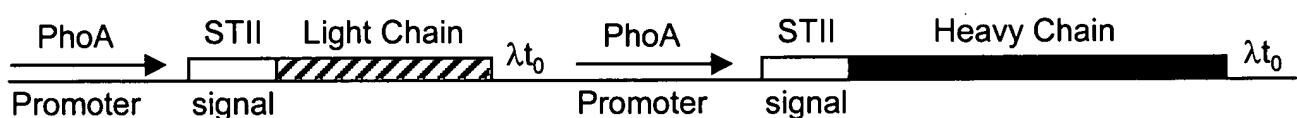


Fig. 3A

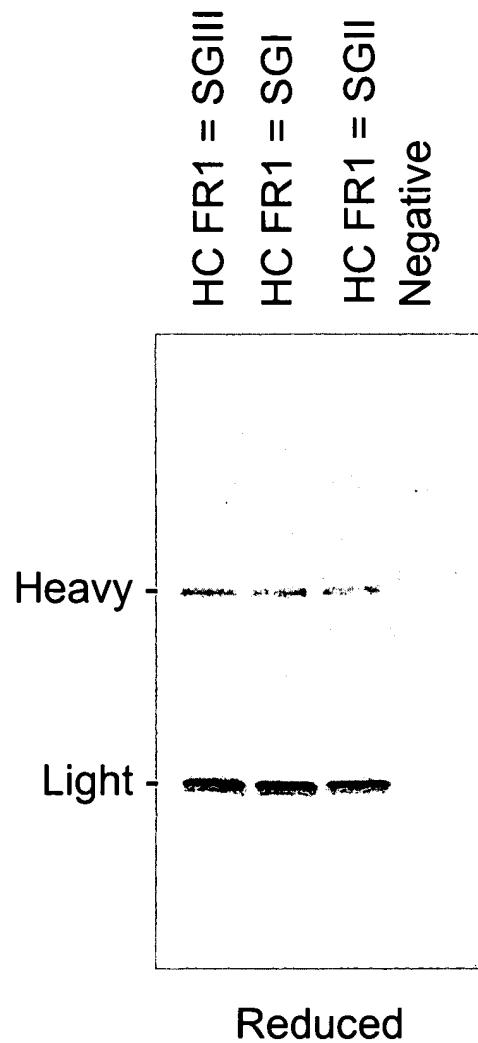


Fig. 3B

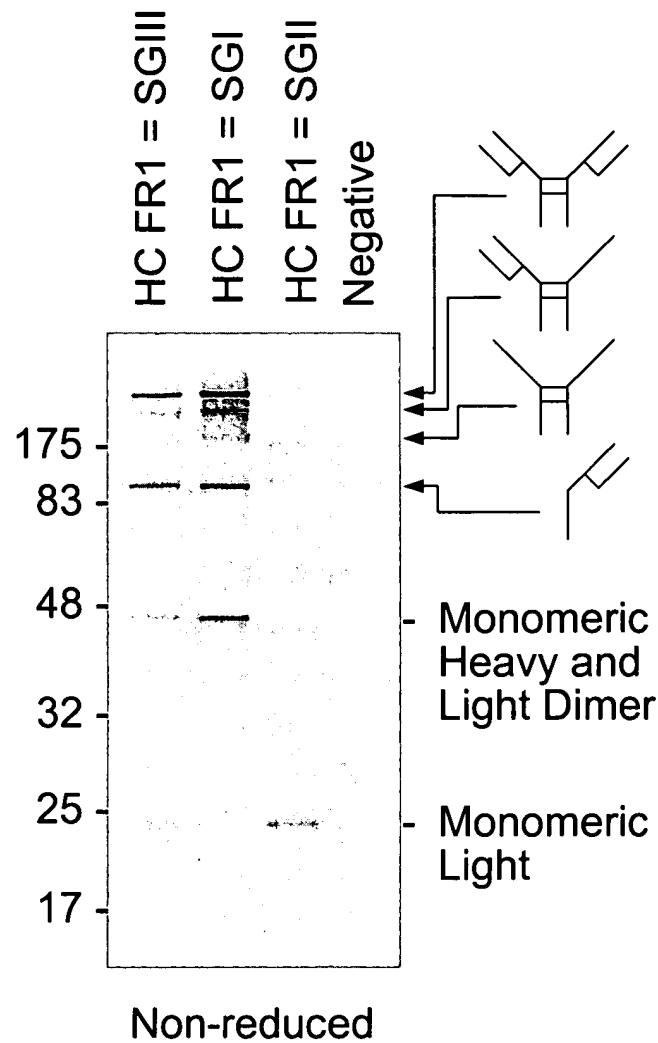


Fig. 4A

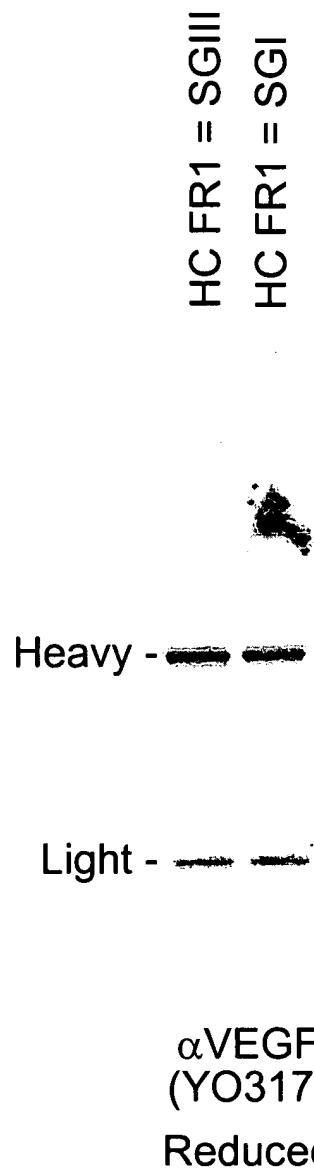


Fig. 4B

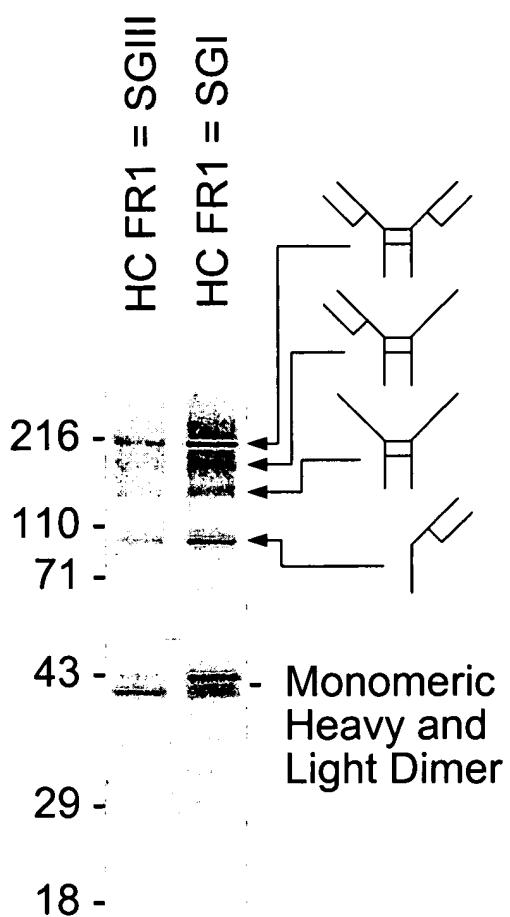


Fig. 5A

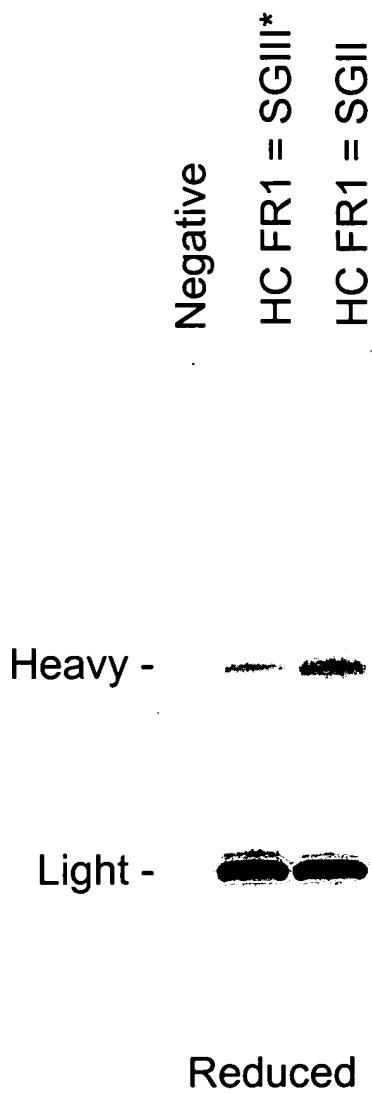
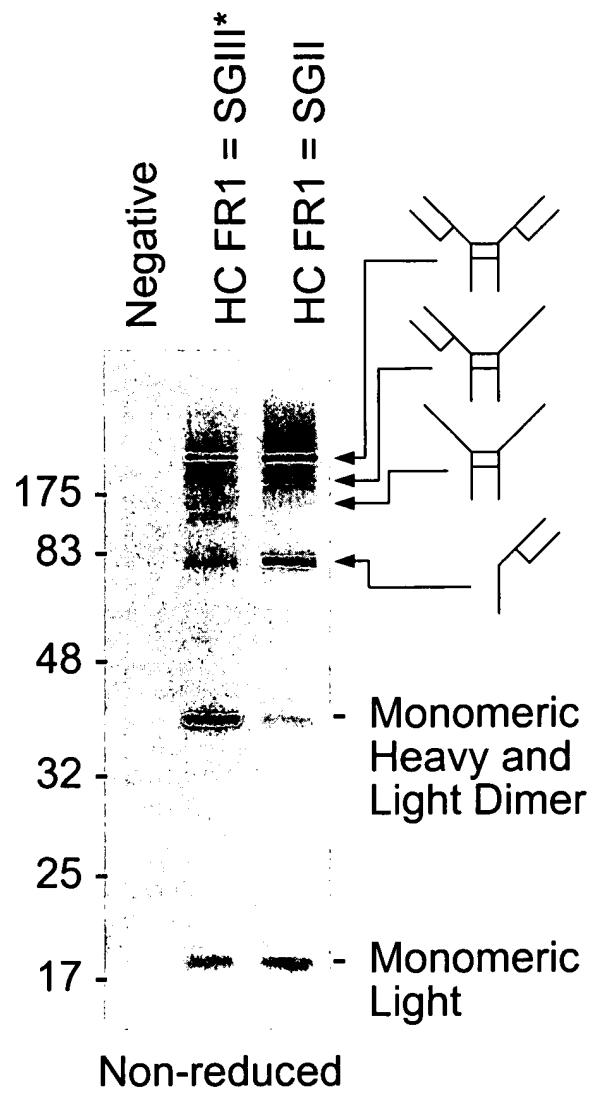


Fig. 5B



*Includes an A24V change as part of humanization.

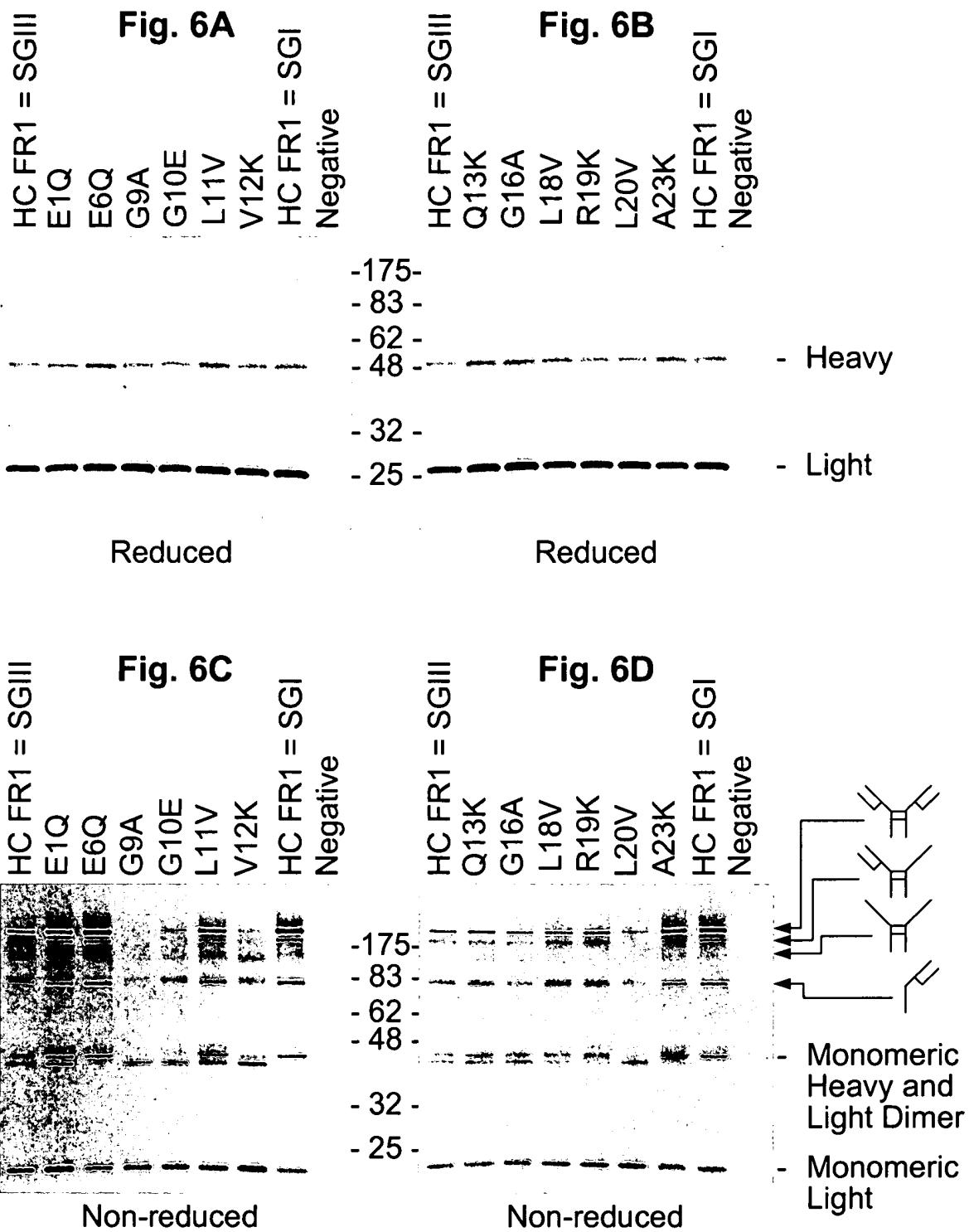


Fig. 7A

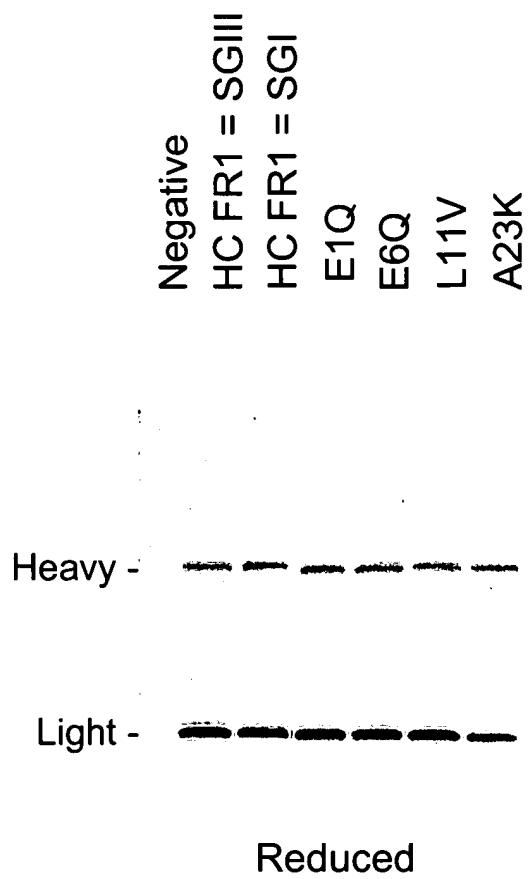


Fig. 7B

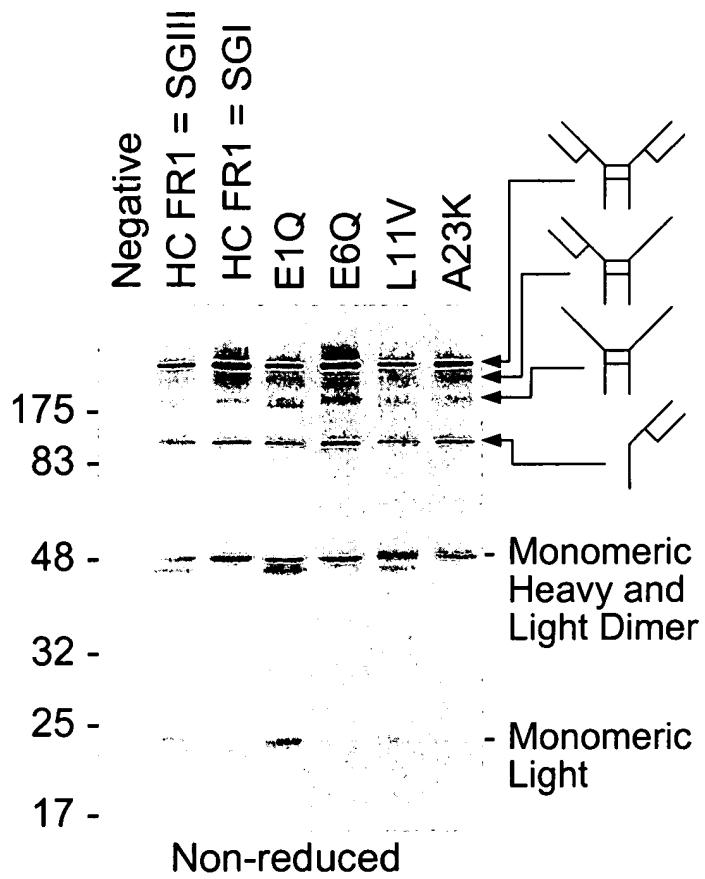


Fig. 8A

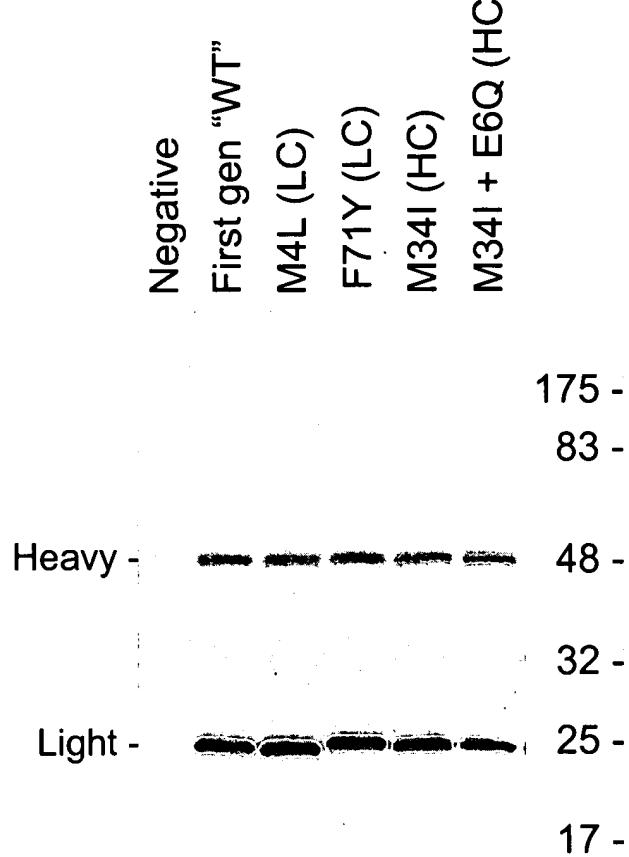


Fig. 8B



Fig. 9A

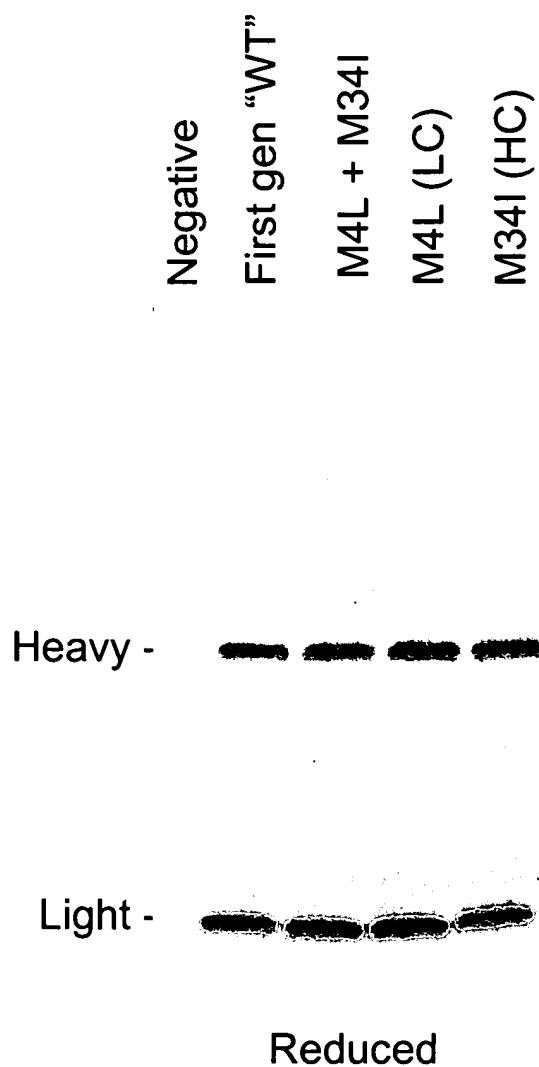


Fig. 9B

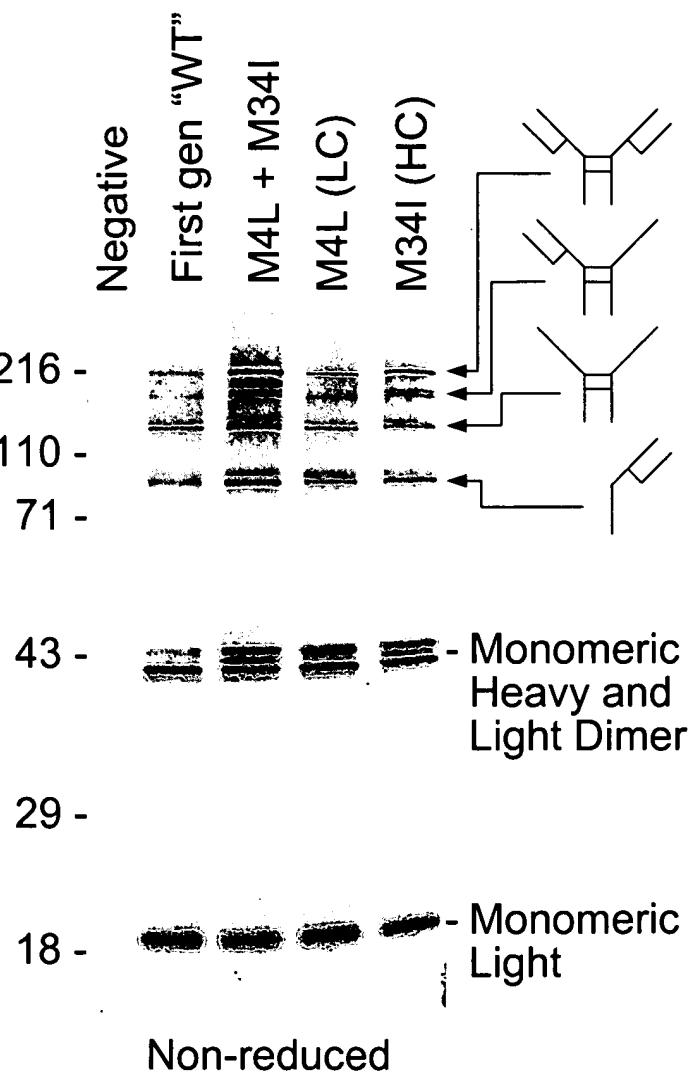


Fig. 10A

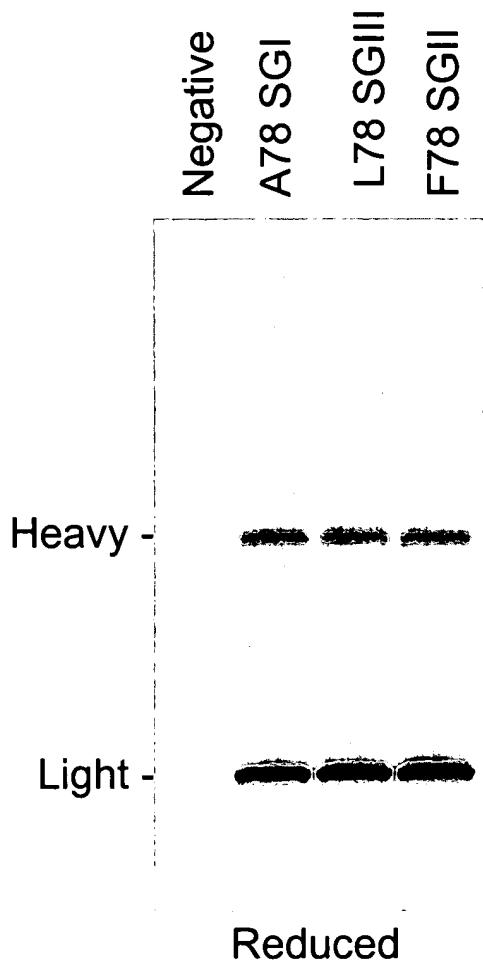


Fig. 10B

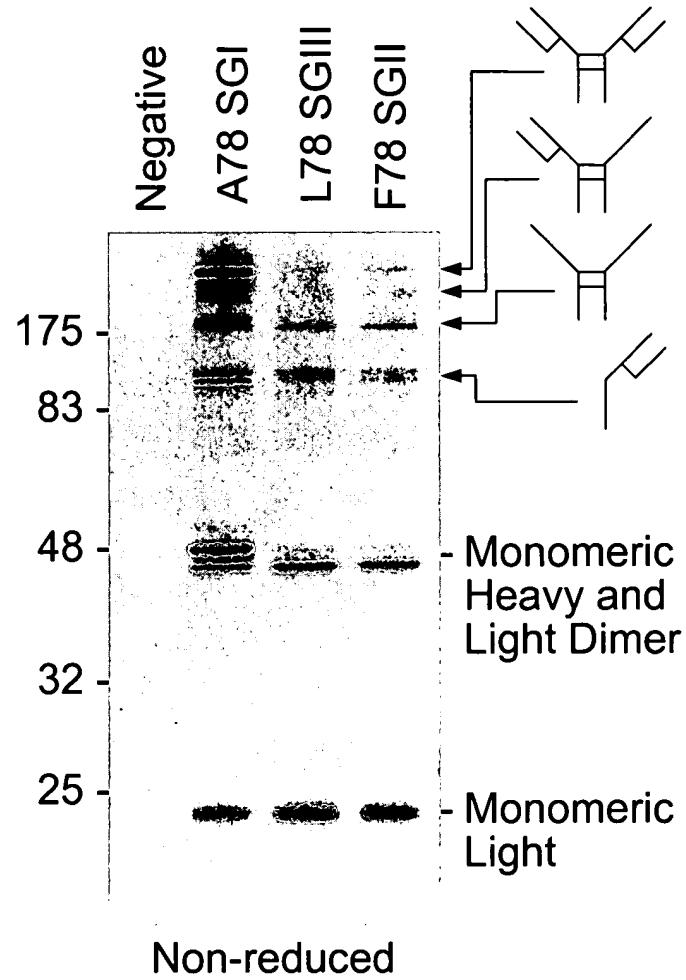


Fig. 11A

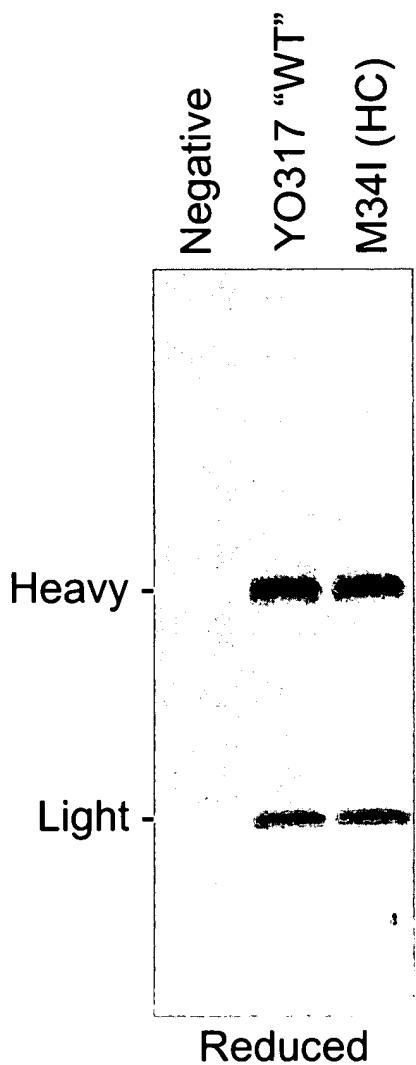


Fig. 11B

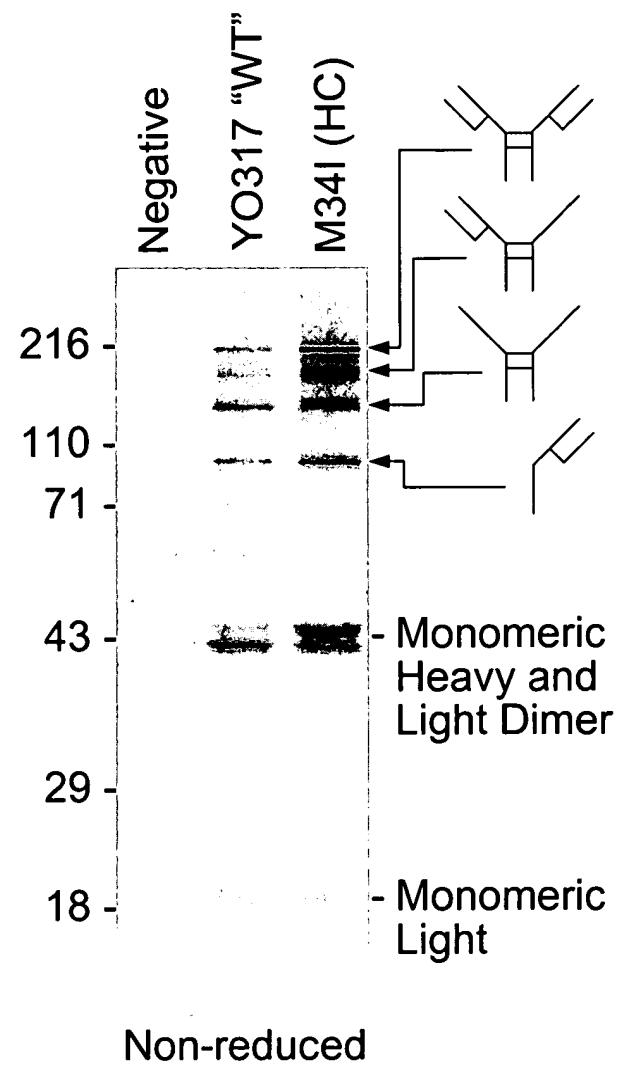


Fig. 12A



Fig. 12B

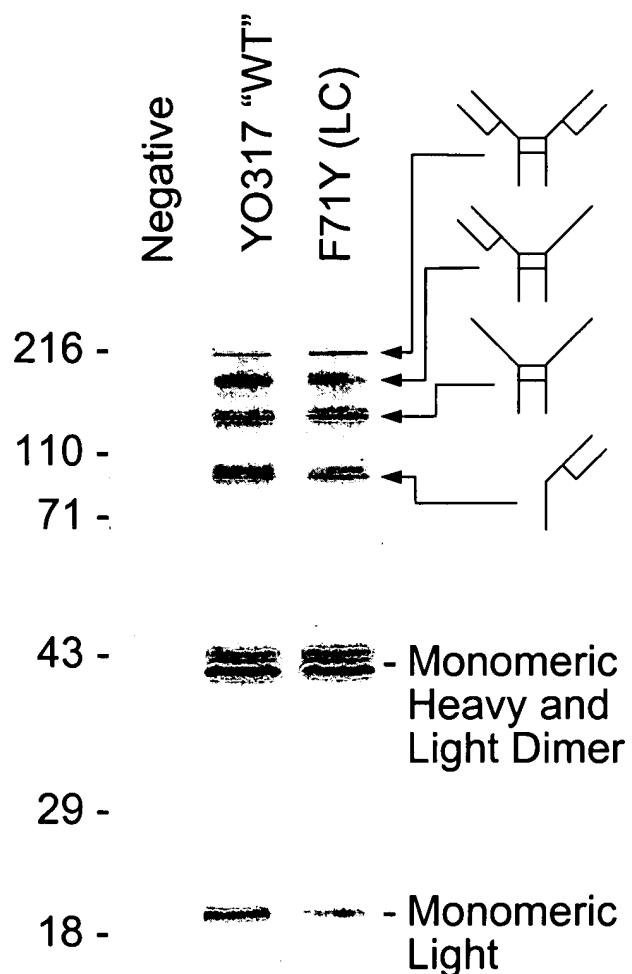
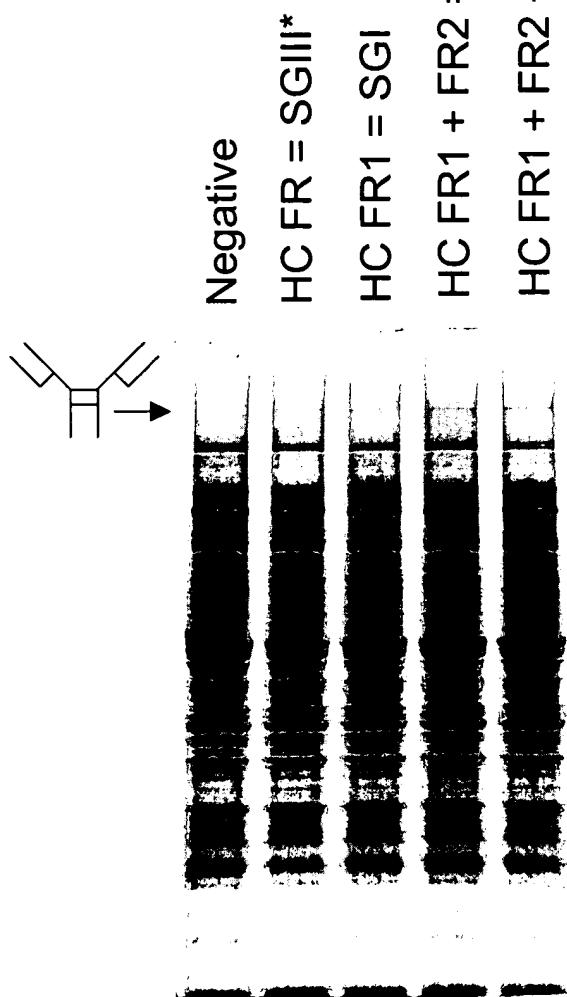
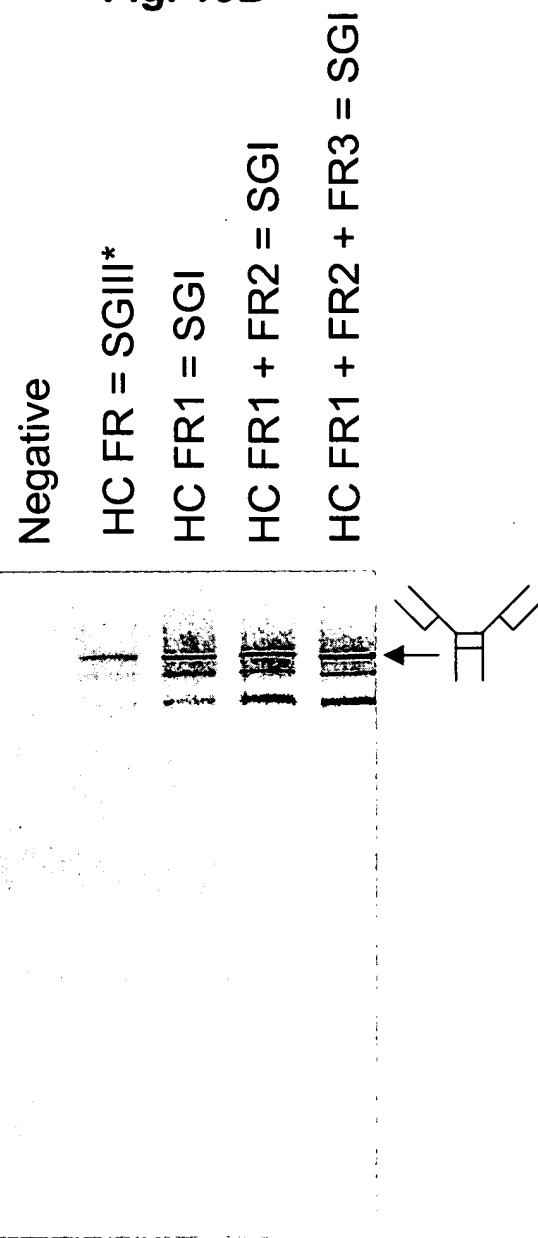


Fig. 13A



Coomassie Stain
Soluble Fraction

Fig. 13B



α Fc Blot
Soluble Fraction

* Except for residues changed during humanization.

Fig. 14A



Fig. 14B

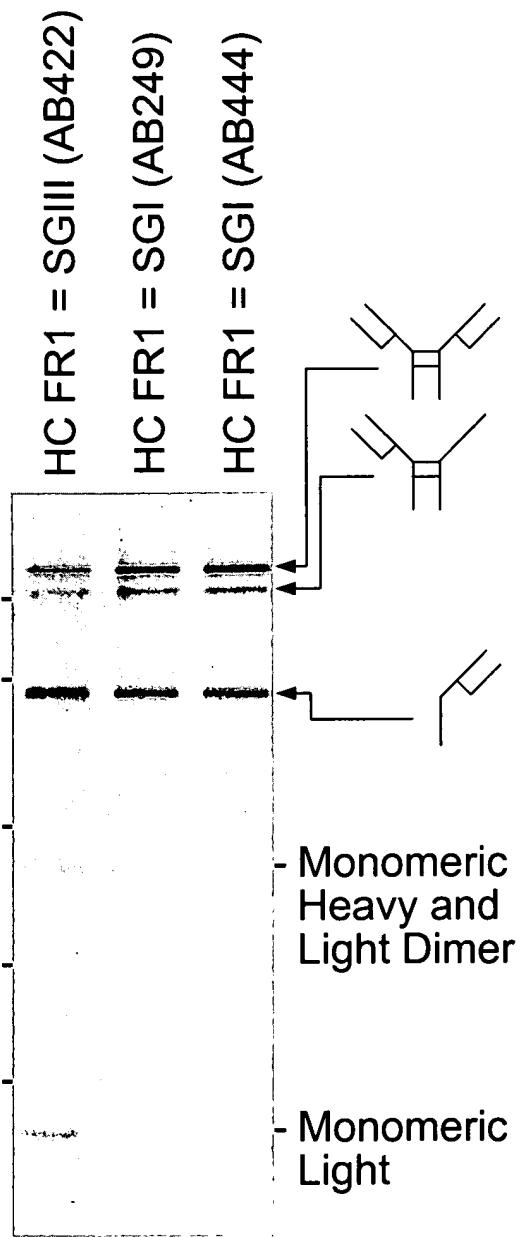


FIG. 15A

Inventor: SIMMONS
Docket No.: 11669.120USU1
Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING
YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE
Attorney Name: KATHERINE M. KOWALCHYK
Phone No.: 612.371.5311
Sheet 15 of 50

1 GAACTCACT TCTCCCACT TTGGATAAGG AAATAACAGAC ATGAAAATC TCATTCGCTGA GTTGTATT AAGCTTGCCC AAAAGAAGA AGAGTCGAAT
CTTAAGTTGA AGAGGTATGA AACCTATTC TTTATGCTCTG TACTTTTAG ACTAAAGACT CAACATAAA TTGAAACGGG TTTTCTCTT TCTCAGCTTA

101 GAACTCTGTG CGCAGGTAGA AGCTTGTGAG ATTATCGCTA CTGCAATGCT TCGAATATG GCGCAAATG GCGCAAATG ACCAACAGGG GTTGATGAT CAGGTAGAGG
CTTGACACAC GCGTCACATCT TCGAAACCTC TAATAGCTAGT GACGTTAGA AGCCTTATAC CGCGTTTAC CGCGTTTAC TGTTGTCGC CAACTAATAA GTTCATCTCC

201 GGGGCGCTGTA CGAGGTAAAG CCCGATGCCA CGCATTCCCTGA CGACGATAGC GAGGTCTGC GGCGATTAGT AAAGAAAGTT TTGAAGCATA CTGTCAGTA
CCCAGCACT GCTCCATTC GGGTACGGT CGTAAGGACT GCTGCTATGC CTGCGACGCG CGCTTATGCA TTTCCTCAT AACTTGGTAG GAGCACTCAT

301 AAAAGTTAAAT CTTTCAACA GCTGTCATAA AGTTGTCACG GCCGAGACCT ATAGTCGCTT TGTGTTTATT TTAAATGTA TTGTAACTA GTAGCAAGT
TTTCAATTAA GAAAAGTTG TGACAGTATT TCAACAGTGC CGGCTCTGAA TATZAGGAA ACAAAATAAA AAAATTACAT AAACATGAT CATGGTTCA

401 TCACGTAATAA AGGGTATCTA GAATTATGAA GAAAATAATC GCATTCTTC TTGCACTCTAT GTTGTGTTT TCTATGCTA CAAACGGTA CGCTGATATC
AGTGCAATT TCCCCATAGAT CTTAATACCTT CTCCTTATAG CGTAAAGAAG AACGTAGATA CAAGGAAAAA AGATAACGAT GTTGGCCAT GCGACTATAG
1 M K K N I A F L L A S M F V F S I A T N A Y A D I
^STII Signal TIR -1

501 CAGTGTACCC AGTCCCCGAG CTCCCTGTCC GGCTCTGTGG GCGATAGGGT CACCATCACC TGAGGGCAA GTCAGGATAAT TAGCAACTAT TAAACTGGT
GTCAACTGGG TCAGGGGTC GAGGGACAGG CGGAGACACC CGCTATCCCA GTGGTAGTGG ACGTGCGTT CAGTCCTATA ATCGTTGATA AATTGACCA
26 Q L T Q S P S L S A S V G D R V T I T C S A S Q D I S N Y L N W Y
^Variable light (VI) cys

601 ATCAACAGAA ACCGGAAA GCTCCGAAG TACTGATTAA CTTCACCTCC TCTCTCCACT CTGGAGTCC TTCTCGCTTC TCTGGATCCG GTTCTGGGAC
TAGTGTCTT TGGTCTTTT CGAGGTCTTC ATGACTAAAT GAATGGAGG AGAGGTGAAGG GACCTAGGG AGACCTAGGC CAAGACCTG
60 Q Q K P G K A P K V L I Y F T S S L H S G V P S R F S G S G S G T

701 GGATTCACT CTGACCATCA GCAGTCTGCA GCCAGAAAGC TTGCAACTT ATTACTGCTCA ACAGTATAGC ACCGTGCGGT GGACGTGTTGG ACAGGGTACCC
CTTAAGTGA GACTGTACT CGTCAAGCT CGGTCTCTG AAGGTTGAA TAATGACAGT TGTCATATCG TGGCACGGCA CCTGCAAACC TETCCCATGG
93 D F T L T I S S L Q P E D F A T Y C Q Q Y S T V P W T F G Q G T
^Variable light cys

801 AAGGTGAGA TCAAACGAAC TGTTGGCTGCA CCATCTGTCT TCATCTGCTT GGCATCTGAT GAGCAGTGA AATCTGGAAC TGCTTCCTGTT GTCTGCTG
TTCCACCTCT AGTTGCTG ACACCGACGT GGTAGACAGA AGTAGAAGGG CGGTGACTA CTGTCACAT TTAGACCTG AGAAAGACAA CACACGGACG
126 K V E I K R T V A A P S V F I F P P S D E Q L K S G T A S V V C L L
Constant Light cys

FIG. 15B

Inventor: SIMMONS
Docket No.: 11669.120USU1
Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING
YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE
Attorney Name: KATHERINE M. KOWALCHYK
Phone No.: 612.371.5311
Sheet 16 of 50

901 TGAATACTT CTATCCAGA GAGGCCAAG TAGCTGGA GGGGATAAAC GCCCTCCAAT CGGGTAACCT CCAGGAGAGT GTCACAGAGC AGGACAGCAA
ACTTATGAA GATAGGTCT CTCCGGTTTC ATGTCACCTT CCACCTATG CGGAGGTTA GCCATTAGAG GGTCCCTCTCA CAGTGTCTCG TCCTGTGTT
160 N N F Y P R E A K V Q W K V D N A L Q S G N S Q E S V T E Q D S K

1001 GGACGGACC TACAGCTCA GCAGCACCCCT GACGCTGAGC AAAGCAGACT ACCGAAACA CAAGCTCAC GCCTGGAAG TCACCCATCA GGGCTGAGC
CCTGTCGGG ARGTCGAGT CGTCGACTCG TTGCGACTCG TTGCTCTGAG TGTTCAGATG CGGAGCTCTC AGTGGTAGT CCCGGACTCG
193 D S T Y S L S S T L T L S K A D Y E K H K V Y A C E V T H Q G L S
^Constant Light cys

1101 TCGCCCGTCA CAAGAGCTT CAAACAGGGGA GAGTGTATTAAATCTCT ACGCGGAGC CATCGGGTAC CGGGGAGT AGGCTAACG
AGCGGGAGT GTTTCGAA GTTGTCCCT CTCACAATTAA ATTAGGAGA TGCGGCTGC GTAGCACGC TGAGGCCATG GGCCTCTAGA TCCGGATTGC
226 S P V T K S F N R G E C O
^cys to bind heavy

1201 CTCGGTTGCC GCCGGGGTT TTATTTGT GCGGACGGG ATCTCGAATG AACTGTGTC GCAGGTAGAA GCTTGGAGA TTATGTCAC TGCAATGCTT
GAGGCTAACGG CGGGCGAA AAAATACAA CGGCTGCGGG TAGAGCTAC TTGACACAGC CGTCCATCTT CGAAACCTCT AATAGCAGTG ACGTTACGAA
^end 1 lambda to terminator

1301 CGCAATATGG CGAACATGAA CCAACAGGG TTGATTGATC AGGTAGAGGG GGGCGTGTAC GAGGTAAAGC CCGATGCCAG CATTCCGTAC GACGATAAGG
GGGTATACCC GCGTTTACT GGTGTCGCC AACTAATAG TCCATCTCC CGCGACATG CTCCATTCTG GGCTACGCTC GTAAAGGACTG CTGCTATGCC

1401 AGCTCTGCG CGATTAGTAA AAGAAAGTAT TGAAGCATCC TCGTCAGTAA AAAGTTAACACAG CTGTCAATA GTGTCACGG CGAGAGCTTA
TCGCGACGC GCTAATGCTAT TTCTTCATA ACTTCGTTAGG AGCAGTCATT TTCAATTAG AAAAGTGTCA GACAGTATT CAACAGTGCC GGCTCTGAAT

1501 TAGTGCTT GTTTTATT TTAATGTAT TTGTAACTAG TAGCGAAGTT CACGTAAAAA GGGTATCTAG AATTATGAG AAGAATATCG CATTCTCTT
ATCAGCGAAA CAAANAAA AAATTACATA AACATGATC ATGGCTCAA GTGCTTTT CCGATAGATC TTATATCG TCTTTATAGC GTAAAGAGA
1 ^STII signal TIR-1

1601 TGCATCTATG TTGCTTTT CTATGCTPAC AAACGGGTAC GCTGAGGTTT AGCTGGTGA GTCTGGCGT GGCCTGGTGC AGCGGGGG CTCACTCCGT
ACGTAATAC AAGGAAAAA GATAACGATG TTGCGCATG CGACTCCAACTG GTATAAACCTG GGTGGTCAAG TCGACCACT CAGACCCCA CGGACCCGTA AGGGCTGGA ATGGTTGGA TGGATTAACA
10 A S M F V F S I A T N A Y A E V Q L V E S G G G L V Q P G G S L R
^anti-VEGF heavy chain (VNTR version)

1701 TTGCTCTG CAGCTCTG CTATACCTTC ACCAACTATG GTATAAACCTG GGTGGTCAAG TCGACCACT CAGACCCCA CGGACCCGTA AGGGCTGGA ATGGTTGGA TGGATTAACA
AACAGGACAC GTCGAAAGACC GATATGGAG TGGTGTATAC CATATTGAC CCAGGCGTCC CGGGGGCAT TCCCGGACCT TACCCAACCT ACCTAATTGT
43 L S C A A S G Y T F T N Y G I N W V R Q A P G K G L E W V G W I N T
^Variable Heavy (VH) cys

1801 CCTATACGG TGAAACGACC TATGCTGCG ATTCAAAAGC TCGTTCTTAG ACACCTCCAA AAGCACAGCA TACCTGGAGA TGAAACAGCCT
GGATATGCGG ACTTGCTCG ATAGGACGC TAAAGTTCG AGCAAAAGTGA AAAAGAATC TTGTTGAGTGT ATGGACGTCT ACTTGCTGGA
77 Y T G E P T Y A A D F K R R F T F S L D T S K S T A Y L Q M N S L

FIG. 15C

Inventor: SIMMONS
Docket No.: 11669.120USU1
Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING
YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE
Attorney Name: KATHERINE M. KOWALCHYK
Phone No.: 612.371.5311
Sheet 17 of 50

1901 GCGCGCTGAG GACACTGCGC TCTATTACTG TCGAAAGTAC CCGGACTATT ATGGAAGCA GCGGAAGAGC CACTGGTATT TCGACGTC TG GGGTCAAGGA
CGCGCAGTC CTGTGACGGC AGATAATGAC AGCTTCTATG GGGCTGATAA TACACTGTCTCG CGCCTTCTCG GTGACCATAA AGTGCAGAC CCCAGTTCTCT
110 R A E D T A V Y Y C A K Y P H Y Y V N E R K S H W Y F D V W G Q G
^VH cys

2001 ACCCTGGTCA CGGTCTCCTC GGCGTCCACC AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAAGA GCACCTCTGG GGGCACAGCG GCCCTGGGCT
TGGGACCGT GGCAAGGGAG CGGAGGGAG CCGGAGGGT TTCCGGGTA GCGAGAAGGG GGACCCGGGG AGGAGGTTCT CGTGGAGACCC CCCGGTGTGCG CGGGACCCGAG
143 T L V T V S S A S T K G P S V F P L A P S S K S T S G T A A L G C
Constant Heavy 1 (CH1) cys^

2101 GCGCTGGTCA GGACTACTTC CCCGAACCGG TCGGGACTCTCA GGCGCCCTGA CCAGCGGGCT GCACACCTTC CGGGCTGTCC TACZGTCCTC
CGGACCAAGT CCGTGAAG GGGTGGCC ACTGCAACG CACCTGAGT CCGGGGACT GTTGTGCGA CGTGTGGAAAG GGCGACAGG ATGTCAGGAG
177 L V K D Y F P E P V T V S W N S G A L T S G V H T F P A V L Q S S

2201 AGGACTCTAC TCCCTCAGCA GCGTGGGTGAC TGTGGCTCTAG AGGAGCTTGG GCACCCAGAC CTACATCTGC AACGTGAATC ACAAGCCAG CAACACCAAG
TCCCTGAGTG AGGGAGTCTG CGCAACCTG ACAGGGAGA TCGTGAACC CGTGGCTCTG GATGTAGACG TTGCACTTAG TTGTCGGTC GTTGGTTC
210 G L Y S L S S V V T V P S S L G T Q T Y I C N V N H K P S N T K
^CH1 cys

2301 GTGGACAGA AAGTGGGCC CAAATCTGT GACAAACACT ACACATGCC ACCCTGCCA GCACCTGAAAC TCCTGGGGGG ACCGTCAGTC TTCTCTTCC
CACCTGTTCT TTCAACTCTGG GTTGTAGACA CTGTTGTGAG TGTGTAGGG TGGCAGGGT CGTGGACTTG AGGACCCCCC TGGCAGTCAG AAGGAGAAGG
243 V D K K V E P K S C D K T H T C P P C P A P E L L G G P S V F L F P
^cys to bind light chain ^hinge cys

2401 CCCCAAAAC CAAAGGACACC CTCATGATCT CCGGACCCCC TGTGCTACAA TGCGTGGTGG TG GACGGTGTAG CCACGAAGAC CCTGAGGTCA AGTCAACTG
GGGGTTTG GTTCTGTGG GAGTACTAGA GGGCTGGGG ACTCCAGTGT ACGCACCAC ACCTGCACTG GGTGCTTCTG GGACTCAGT TCAAGTTGAC
277 P K P K D T L M I S R T P E V T C V V V D V S H E D P E V K F N W
^Constant Heavy 2 (CH2) cys

2501 GTACGTGGAC GGCCTGGAGG TGACATAATGC CAAGACAAAG CGCGGGAGG AGGAGTACAA CAGCACGTAC CGTGTGGTCA GGGTCTCAC CGTCTGCAC
CATGCACTG CGGCACCTCC ACGATTACG GTTCTGTTC GGGCCCTCC TCGTCATGTT GTCTGTGATG GCACACAGT CGCAGGAGTG GCAGGACGCTG
310 Y V D G V E V H N A K T K P R E E Q Y N S T Y R V V S V L T V L H

2601 CAGGACTGSC TGAATGGCA AGGAGTACAAG TCGAAAGGTCT CCTACAAAGC CCTCCCAAGCC CCACATCGAGA AAACCATCTC CAAAGCAAA GGGAGCCCC
GTCCGTGACCG ACTTACCGTT CCTCATGTTT CGTGTGTTCG GGAGGGTGG GGTAGCTT TTGGTAGAG GTTTCGGTT CCCGTGGGG
343 Q D W L N G K E Y K C K V S N K A L P A P I E K T I S K A . K G Q P R
^CH2 cys

2701 GAGAACCACA GTGTGACACC CTGCCCCCAT CCGGGAAAGA GATGACCAAG AACCGGTCAA GCTGTGACCTG CCTGGTCAA GGCTTCTATC CCAGGACAT
CTCTTGGTGT CCACATGTGG GACGGGGTA GGGCCCTCTCT CTACTGGTCT TTGGTCCAGT CGGACTGGAAC GGACCAAGTTT CGGAAGATAG GTTGGCTGTA
377 E P Q V Y T L P P S R E E M T K N Q V S L T C L V K G F Y P S D I
^Constant Heavy 3 (CH3) cys

FIG. 15D

Inventor: SIMMONS
Docket No.: 11669.120USU1
Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING
YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE
Attorney Name: KATHERINE M. KOWALCHYK
Phone No.: 612.371.5311
Sheet 18 of 50

2801 CGCCCGTGGAG TGGGAGGCA ATGGGAGGCC GGAGAACAAAC TACAAGACCA CGGCCCTCCGT GACGGACTCC GCTGGACTCC TCTTCCCTCTA CAGGAAGCTC
410 GCGGGACCTC ACCCTCTCGT TACCCGTCGG CCTCTTGTGG ATGTTCTGGT GCGGGAGGA AGAAGGAGAT GTGGTTCGAG
A V E W E S N G Q P E N N Y K T T P P V L D S D G S F F L Y S K L

2901 ACCGGGAGCA AGAGGAGGTG GCAGGAGGG AACGTCCTCT CAGTCCTCGT GATGATGAG GCTCTGACA ACCACTAAC GCAGAAAGAGC CTCTCCCTGT
443 T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L S L S
TGGCACCTGT TCTCGTCCAC CGTCTGGGAT CTCAGGAGCA GTACGAGGCA CTACGTAATC CGAGACCTGT TGGTGTATGT CGTCTTCTCG GAGAGGGACA
^CH3 cys

3001 CTCCGGTAA ATAAGCATGC GACGGCCCTAA GAGTCCTAA CGCTGGTTG CCGCCGGCG TTTTTATG TTAACTCATG TITGACAGCT TATCATCGAT
477 P G K O ^start lambda to terminator ^end lambda to terminator ^start of tet resistance promoter ^-35 of promoter
GAGGCCATT TATTCTGAG CTGCGGGAT CTCAGGGATT GCGAGCCAAAC GGGGGCCGC AAAAATAAC AATTGAGTAC AAACTGTGCA ATAGTAGCTA

3101 AAGCTTAAAT GCGGTAGTTT ATCACAGTTA ATTGCTAAC GGAGTCAGGC ACCGTGTATG AAATCTAAC ATGGCTCAT CGTCATCCTC GGCACCGTCA
TTGGAAATTA CGCCATCAA TAGTGTCAAT TTAACGATTG CGTCAGTCCG TGGCACATACT TTAGATGT TAGGCGAGTA GCAGTAGGAG CGTGGCAGT
^ -10 region of tet resistance promoter ^ start of tet resistance translation

3201 CCCCTGGATC TGTAGGCATA GGCTTGGTAA TGCGGGTACT GCCGGGCTC TTGGCGGATA TCGTCCATTIC CGACAGGATC GCCAGTCACT ATGGGTGCT
GGGACCTAG ACATCCGAT CGAACCAAT CGAACCATGA CGGCGGAG AACGCCCTAT AGGAGGTAAAG GCTGTGAG CGGTCACTGA TACCGCACGA

FIG. 16A

Inventor: SIMMONS
Docket No.: 11669.120USU1
Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING
YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE
Attorney Name: KATHERINE M. KOWALCHYK
Phone No.: 612.371.5311
Sheet 19 of 50

1 GAATTCAACT TCTCCATACT TTGGATAAGG AAATACAGAC ATGAAAATC TCAATTGCTGA GTTGTATT AAGCTTGCCC AAAAGAAGA AGACTCGAAT
CTTAAAGTTGA AGAGGTATGA AACCTATTC TTAGTGTCTG TACTTTAG AGTAAGACT CAACAATAAA TTGAAACGGG TTTTCTCTT TCTAGCTTA
101 GAACTGTG CGCAGGTAGA AGCTTGGAG ATTATCGTC CTGCAATGCT TCGCAATATG GCGCAAATG ACCAACAGCG GTTGATGTGAT CAGGTAGAGG
CTTGACACAC GGGTCATCT TCGAAACCTC TTAAGGACT GACGTTAGCA AGCTTATAC CGGGTTAAC TGGTGTGCGC CAACTAACTA GTCCATCTC
201 GGGCGCTGTA CGAGGAAAG CCCGATGCCA GCATTCCTGCA CGACGATACG GAGCTGCTGC CGGATTACGT AAAGAAGITA TTGAAGCATC CTGCGTCAGTA
CCCCGGACAT GCTCCATTTC GGGCTACGGT CGTAAGGACT GCTGCTATGC CTCGACGACG CGCTAAATGCA TTCTCTCAAT AACCTGTAG GAGGAGTCAT
301 AAAAGTTAAT CTTTCACCA GCTGTCATAA AGTTGTCAAG GCCGAGACTT ATAGTGCTT TGTTTTATT TTTAAATGTA TTGTAACTA GTACCGAAGT
TTTTCATTA GAAAGTGT CGACAGTATT TCAACAGTGC CGGCTCTGAA TATCGCGAA ACAAAATAA AAAATTACAT AAACATGTAT CATCGTTCA
401 TCACGTAAGG AGGTTACCA GAAATTGAA GAAAGAATATC GCATTTCCTTC TTGCACTAT TGTCTTTTT TCTATTGCTA CAAACGGTA CGCTGATATC
AGTGCAATTTC TCCCATTAGT CTTAAACTCTT CTTCTTTAGT CGTAAAGAAG AACGTAGATA CAAGGAAAAA AGATAACGAT GTTGGCGCAT GCGACTATAG
1 M K K N I A F L L A S M F V F S I A T N A Y A D I
^STII signal TIR ~1 Anti-VEGF Light chain (version Y0317)~
501 CAGTGCACC AGTCCCCGAG CTCCCTGTC GCCTCTGTG GCGATAGGGT CACCATCAC TGAGCGCIA GTAGGATAT TAGCAACTAT TPAACTGGT
GTCAACTGGG TCAGGGCTC GAGGGACAGG CGGAGACACC CGCTATCCCA GTGTTAGTGG AGCTCGCGTT CAGTCCTATA ATCGTTGATA AATTTGACCA
26 Q L T Q S P S L S A S V G D R V T I T C S A S Q D I S N Y L N W Y
601 ATCAACAGAA ACCAGGAAA GCTCCGAAAG TACTGATTTC CTCACCTCC TCTCTTCACT CTGGAGTCCC TTCTCGCTTC TGCTGATCCG GTCTGGAC
TAGTGTCTT TGGCTCTT CGAGGCTTC ATGACTAAAT GAAAGGGAGG AGAGGGTGA GACCTCAGGG AAGAGCGAAG AGACCTAGGC CAAGACCTG
60 Q Q K P G K A P K V L I Y F T S S L H S G V P S R F S G S G S G T
701 GGATTCACCT CTGACCATCA GCGAGTCGCA GCCGAGAGAC TTGGCAGCTT ATTACTCTCA ACAGTATGC ACCGTGCGGT GGACGTTGG AGACGGTAC
CCTAAAGTGAA GACTGGTAGT CGTCAAGCTG CGGTCTTC TAATGACAGT TGTCATATCG TGGCACGGCA CCTGCAAACC TGTCCTATGG
93 D F T L T I S S L Q P E D F A T Y Y C Q Q Y S T V P W T F G Q G T
801 AAGGTGAGA TCAAACGAAAC TGTGGCTGCA CCATCTGTCT TCACTCTCC GCCATCTGAT GAGCAGTGA AATCTGGAAC TGCTCTGTT GGTGCGCTGC
TTCCACCTCT AGTTGGCTG ACACCGACGT GGTAGACAGA AGTAGAAGGG CGGTAGACTA CTCGTCACAT TTGACCTG AGGAAGACAA CACGGGACG
126 K V E I K R T V A A P S V F I F P P S D E Q L K S G T A S V V C L L
901 TGAATAACTT CTATCCAGA GAGGCCAAAG TACAGTGGAA GTTGTGATAAC GCCCTCCAAT CGGGTAAC CCAGGAGGT GTCACAGAGC AGGACAGCAA
ACATTGAA GATAGGGTCT CTCGGGTTTC ATGTCACCTT CCACCTATTG CGGGAGTTA GCCCAATTGAG GGTCCTCTCA CAGTGTCTG TCCMTCGTT
160 N N F Y P R E A K V Q W K V D N A L Q S G N S Q E S V T E Q D S K

FIG. 16B

Inventor: SIMMONS
Docket No.: 11669.120USU1
Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING
YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE
Attorney Name: KATHERINE M. KOWALCHYK
Phone No.: 612.371.5311
Sheet 20 of 50

1001 GGACAGCAC TACAGCCCTCA GCAGCACCCCT GACGCTGAGC AAAGCAGACT ACCGAAACA CAAAGCTCAC GCCTGCGAAG TCACCCATCA GGGCTGAGC
CCTGCTGGTGG ATGTCGGAGT CGTCTGCGGA CTGCGACTCG TTTCGACTCG TTTCGCTCTGA TGCTCTTGT GTTCTCAGATG CGACGCTTC AGTGGTAGT CCCGACTCG
193 D S T Y S L S S T L T L S K A D Y E K H K V Y A C E V T H Q G L S

1101 TCGCCCGTCA CAAGAGCTT CAACAGGGGA GAGGTAAAT TAATCTCT ACGGCGAGG CATCGTGGAC AGCTCGGTAAC CGGGGATCT AGGGCTAACG
AGCGGGCAGT GTTCTCGAA GTTGTCCCT CTCACAATTA ATTAGGAGA TGCGGCTGC TGAAGCCATG GGAGCCATG TCCGATTGA TCCGGATTGC
226 S P V T K S F N R G E C O

1201 CTCGGTTGCC GCCGGGGCTT TTTATTGTT GCCGACGCGC ATCTCGAATG AACTCTGTC GCAGGGTAGA GCTTTGGAGA TTATGTCAC TGCATATGCTT
GAGCCAACGG CGGCGCGAA AAAATAACAA CGGCGAACAGT TTGACACAGC TAGAGCTAC CGTCCCATCTT CGAAACCTT AATAGCAGTG ACGTAGAA

1301 CGCAATATGG CGCAAAATGA CCAACAGGG TTGATTGATC AGGTAGAGGG GGGCGCTSTAC GAGGTAAAGC CGATGCCAG CATTCTGAC GACGATACGG
GGGTATACCC GGGTTTACT GGTGTGCGC ACTRACTAG TCCATCTCCC CGCGACATG CTCCATTCTG GGCTACGGTC GTAAGGACTG CTGCTATGCG

1401 AGCTCTCTCG CGATTACGTA AAGGAGTTAT TGAAGCATTC TCGTCAGTTA AAAGGTAATC TTTCAACAG CTGTCAATAA GTTGTACGG CCGAGACTTA
TCGACCGACG GCTAATGCT TTCTTCATAA ACTTCGTTAGG AGGAGCTATT TTCTCAATT AAAGGTTGTC GACAGTATT CAACAGTGCC GGCCTGAAAT
1501 TAGTCGCTT GTTTCATT TTAATGTT TTGTAATCTAG TACGAAAGT CACGTAAAAA GGTATCTAG ATTATGAG AGAAATTCG CATTCTCTT
ATCAGCGAA AAAATAAA AAATACATA ACATTGATC ATGGCTCAA GTGCAATTTC CCATAGATC TTAATACTC TCTTTATAGC GTAAAGAAGA
1 M K K N I A F L L
^STII signal TIR-1

1601 TGCATCTATG TTTCGTTTTT CTATTGCTAC AAACGCGTAC GCTGAGGGTT AGCTGGTGA GTCTGGGGT GGCCCTGGTGC AGCCAGGGG CTCACTCCGT
ACGTAGATAC AAGCAAAA GATAACGATG TTTCGCGATG CGACTCCAAG TCGACCACT CAGACGCCA CGGACCCAG TCGTCCCCC GAGTGGCCA
10 A S M F V F S I A T N A Y A E V Q L V E S G G L V Q P G G S L R
^Anti-VEGF Heavy Chain (version Y0317)

1701 TTGTCTCTG CAGCTCTGG CTAGCACTTC AGGCACTACG GTATGAACTG GGTCCCTCAG GCCCGGGTA AGGGCTGGA ATGGGTGGA TGGATTAACA
AACAGGACAC GTCGAAGAC ACTTGGCTGG ATACGACGCC TAAAGTTGC AGCAAGTGA AAAAGAATC TGGGAGGTI TTGTGTGT ATGGAGCT ACTTGTGGA
43 L S C A A S G Y D F T H Y G M N W V R Q A P G K G L E W V G W I N T
77 Y T G E P T Y A A D F K R R F T F S L D T S K S T A Y L Q M N S L
^Anti-VEGF Heavy Chain (version Y0317)

1801 CCTATACGG TGAAACGGC TAGTATTACTG TGCAAAGTAC TTTCGTTCACT GCACTGGTAT TTGACAGCA TACCTCCAA AGGCACAGCA TACCTGAGA TGACAGCCT
GGATATCGG GACACTGGC TCTATTACTG TGCAAAGTAC CCGTACTATT ATGGGACGAG CCACTGGTAT TTGACAGTCT GGGCTGGTGC AACCCCTGGTC
43 L S C A A S G Y D F T H Y G M N W V R Q A P G K G L E W V G W I N T
77 Y T G E P T Y A A D F K R R F T F S L D T S K S T A Y L Q M N S L
^Anti-VEGF Heavy Chain (version Y0317)

1901 GCGCGCTGAG GACACTGGC TCTATTACTG TGCAAAGTAC CCGTACTATT ATGGGACGAG CCACTGGTAT TTGACAGTCT GGGCTGGTGC AACCCCTGGTC
CGCGCGACTC CTGTGACGCC AGATAATGAC ACGTGCTCATG GGCATGATAAA TACCCCTGCTC GTGACCCATA AAGCTGAGA CCCAGTTCC TTGGGACCAAG
110 R A E D T A V Y C A K Y P Y Y G T S H W Y F D V W G Q G T L V
^Anti-VEGF Heavy Chain (version Y0317)

2001 ACCGCTCTCT CGGCCCTCCAC CAAGGGCCA TCGCTCTTC CCCTGGGACCT CGACACCTCTG GGGGACAGC GGCGCTGGGC TGCTCTGGTCA
TGGCAGAGGA GCGGGAGGT GTTCCGGGT AGCCAGAAGG GGGACCGTGG CCGGGACCCG ACGGACCAAG
143 T V S S A S T K G P S V F P L A P S S T S G G T A A L G C L V K

FIG. 16C

Inventor: SIMMONS
 Docket No.: 11669.120USU1
 Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING
 YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE
 Attorney Name: KATHERINE M. KOWALCHYK
 Phone No.: 613.371.5311
 Sheet 21 of 50

2101 AGGAACTACTT CCCCGAACCG GTGAGGGTGT CGTGAACACT ACCAGGGCTT CGGAGCTCTT CCCGGCTCTT CTACAGCTCTT CAGGACTCTA
 177 TCTGTGAA GGGGCTGGC CACTGCTCAA GCACTGCTAG TCCGCGGAC ACGTGTGAA GGCGCGACAG GATGTGAGGA GTCTCTGAGAT
 2201 CTGCCCTAGC AGCGCTGGTA CTGTCCTCTC TAGCAGCTTG GCCACCCAGA CCTACATCTG CAACGTGAAT CACAAGCCCA GCAACACCAA GTGGACAAAG
 210 S L S S V V T V P S S L G T Q T Y I C N V N H K P S N T K V D K
 2301 AAAGTGAGC CCAAATCTTG TGACAAACT CACACATCC CACCGTCCC AGCACCTGAA CTCCTGGGG GACGTCAGT CTTCTCTTC CCCCAAAAC
 243 K V E P K S C D K T H T C P P C P A P E L L G G P S V F L F P P K P
 2401 CCAGGACAC CCTCATGATC TCCCAGACCC CTCAGGTAC AGTCGCTGTG GTGGAGGTGA GCCACGAAAGA CCCTGAGCTC AAGGTCAGT GGTAGCTGGA
 277 K D T L M I S R T P E V T C V V V D V S H E D P E V K F N W Y V D
 2501 CGCGCTGAG GTGCAATATG CCAGACAAAGA GCGCGGGAG GAGCGATACA ACAGCGCTA CCGAGCTGTA AGCGCTCTCA CGTGTCTGCA CGAGACTGGA
 310 G V E V H N A K T K P R E E Q Y N S T Y R V V S V L T V L H Q D W
 2601 CTGAATGCA AGGAGTACAA GTGCAAGGTG TCGAACAGAG CCCTCCAGC CCCCATCGAG AAACACATCTT CCAAGGCAA AGGGGGCCC CGAGAACAC
 343 L N G K E Y K C V S N K A L P A P I E K T I S K A K G Q P R E P Q
 2701 AGGTGTACAC CCTGCCCCCA TCCCGGGAG AGATGACCAA GAACCGAGTC AGCCCTGACCT GCTCTGCTCA AGGCTCTAT CCCAGGACA TCGCGCTGGA
 377 V Y T L P P S R E E M T K N Q V S L T C L V K G F Y P S D I A V E
 2801 GTGGGAGAGC ATGGGCAAGC CGGAGAACCA CTAAAGAC AGGCCCTCGG TGCTGGACTC CGAGGCTCC TTCTCTCT ACAGGAAGCT CACCTGGAC
 410 W E S N G Q P E N N Y K T T P P V L D S D G S F F L Y S K L T V D
 2901 AACAGGAGGT GCGAGGAGG GAAACCTCTC TCATGCTCCG TGATGCTGAA GAAACACTACA CGCTCTGCA CCTCTCTCTG TCTCTGGGTA
 443 K S R W Q Q N V F S C S V M H E A L H N H Y T Q K S L S L S P G K
 3001 AATAAGGATG CGACGCCCT AGAGTCCCTA ACGCTCGTT GCGGCCGGG GTTTTATT GTAACTCAT GTTGACAGC TTATCATCGA TAAGCTTAA
 477 O

3101 TGGGTAGTT TATCAGATTA AAATTGCTAA CGAGCTGAGG CACCGTGTAT GAAATCTAAC AATGCGCTCA TGGTCATCTT CGCACCGTC ACCCTGGATG
 ACCCCATCAA ATAGCTCAA TTAACTGATT GCCTGCTTCG GTGGCACATA CTTAGATGTT TTACCGAGT AGGATGAGA GCGCTGGCAG TGGACACTAC
 ^Start Ter Resistance Coding Sequence

FIG. 16D

3201 CTGTAGGCAT AGGCCTGGTT ATGCCGGGTAC TGGCGGGCCT CTGGCGGAT ATCCGTCCATT CGCACAGCAT CGCCAGTCAC TATGGCGTGC TGGCTAGCGCT
GACATCCGTA TCCGAACCAA TACGGCCATG ACGGGCCCCAA GAACGCCCTAA TAGCAGGTA GGCTGTCCGTA GCGGTCAAGTG ATACCGCAGC ACGATCGCGA

FIG. 17A

Inventor: SIMMONS
Docket No.: 11669.120USU1
Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING
YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE
Attorney Name: KATHERINE M. KOWALCHYK
Phone No.: 612.371.5311
Sheet 23 of 50

1 GAATTCACT TCTCCACT TTGATAAGG AATACAGAC ATGAAAAATC TCATTGCTGA GTTGTATT AAGCTTGCCC AAAAGAAGA AGAGTCGAAT
CTTAAGTGA AGAGGTATGA AACTATTC CTTATGTCG TACTTTTAG AGTAAAGACT CAAACATAAA TTGAAACGGG TTTTCTCTT TCTAGCTTA

101 GAACTGTTG CGCAGGTTAGA AGCTTGGAG ATTATCGTCA CTGCAATGCT TCGAAATATG GCGCAAAATG ACCAACAGGG GTTGATGAT CAGGTAGAGG
CTTGACACAC GCGTCATCTC TCGAAACCTC TAATAGCGT GACGTAGCA AGCGTTAC CGCGTTAAC CAACTAACTA GTCCATCTC

201 GGGCGCTGTA CGAGGTAAG CCCGATGCCA GCGATGCCA GCGATGCCA CGAGGATAGC GAGCTGCTGC GGAGTAACTG AAAGAAAGTA TTGAAAGCATC CTCGGTCACTA
CCCCGCACAT GCTCCATTG GGGCTACGT CGTAAAGGACT GTCTGCTATGC CTCGAGGACT CGCTAAATGCA TTTCCTCATC AACCTGTTAG GAGCGATCAT

301 AAAAGTTAT CTTTCAACA GCTGTCAAA AGTTGTCAAGC GCGGAGACTT ATAGTCGCTT TTGTTTATT TTAAATGTA TTGTAACTA GTAGCAAGT
TTTCAATTA GAAAAGTGT CGACAGTATT TCAACAGTGC CGCGCTGAA TATCAGCAGA ACAAAATTA AAAATTACAT AAACATGTAT CATTGTTCA

401 TCACGTAAA AGGGTATCTA GAATTATGAA GAGAATATC GCATTCTTC TTGCACTAT GTTGTGTTT TCTATGCTA CAAACGGTA CGCTGATATC
AGTGCATTTC TCCCATAGAT CTTAATACCTT CTCCTTATAG CGTAAAGAAG AACGTAGATA CAAGCAAAA AGATAACGAT GTTGGCCAT GCGACTATAG
1 M K K N I A F L L A S M F V F S I A T N A Y A D I
^STII Signal TIR -1

501 CAGTTGACCC AGTCCCCGAG CTCCCTGTCG GCGATAGGGT CACCATCACC TGAGCGCAA GTCAAGGATAT TAGCAACTAT TAAACTGGT
GTCAACTGG TCAGGGGTCG GAGGGACAGG CGGAGACACCG CGCTATCCCA GTGGTAGTGG ACGTGCGTT CAGTCCTATA ATCGTTGATA AATTGACCA
16 Q L T Q S P S L S A S V G D R V T I T C S A S Q D I S N Y L N W Y
^Variable light (VL) Cys

601 ATCAACAGAA ACCAGAAA GCTCCGAAAG TACTGATTAA CTTCACCTCC TCTCTCCACT CTGGAGTCCC TTCTCGCTTC TCTGGATCCG GTTCTGGGAC
TAGTGTCTT TGGTCCTTT CGGGCTTC ATGACTAAAT GAACTGGGG AGAGAGGTGA GACCTCAAGG AAGAGCGAAG AGACCTAGGC CAAGACCTG
60 Q Q K P G K A P K V L I Y F T S S L H S G V P S R F S G S G S G T

701 GGATTCACT CTGACCATCA GCAGTCTGCA GCCAGAAAGAC TTGCAACTT ATTACTCTCA ACAGTATGCC ACCGTGCGGT GGACGTTGG ACAGGGTACCC
CTTAAGTGA GACTGGTAGT CGTCAAGCT CGGTCTCTCG AAGGGTTGAA TAATGACAGT TGTCATATCG TGGCACGGCA CCTGCAAACC TGTCCATGG
93 D F T L T I S S L Q P E D F A T Y Y C Q Q Y S T V P W T F G Q G T
^Variable light cys

801 AAGGTGGAGA TCAAACGAAAC TGTGGCTGCA CCATCTGCTC TCATCTGCTC GCCATCTGAT GAGCAGTGA AATCTGGAAAC TGCTCTGTT GTGTCCTGC
TTCCACCTCT AGTTGGCTG ACCAGCAGCT GGTAGACAGA ACTAGAAGGG CGGTGACTA CTGCTCACT TTGACCTTG AGAAAGACAA CACACGGACG
126 K V E I K R T V A A P S V F I F P P S D E Q L K S G T A S V V C I I
Constant Light cys^

FIG. 17B

Inventor: SIMMONS
Docket No.: 11669.120US1
Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING
YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE
Attorney Name: KATHERINE M. KOWALCHYK
Phone No.: 612.371.5311
Sheet 24 of 50

901 TGATAAATT CTATCCAGA GAGGCCAAAG TACAGTGGAA CCGTGGATAAC GGCCTCCAAT CGGGTAACTC CCAGGAGAGT GTCAAGAGC AGGACAGCAA
ACTTATTGAA GATAAGGTCT CTCGGGTTC ATGTCACTT CCACCTATTG CGGGAGGTTA GCCATTGAG GGTCCCTCTCA CAGTGTCTCG TCCCTGTGTT
160 N N F Y P R E A K V Q W K V D N A L Q S G N S Q E S V T E Q D S K

1001 GGACAGGACC TACAGCCTCA GGAGGACCT GACGCTGAGC AAAGGAGACT ACAGAAACA CAAAGTCTAC GCCTGGAAAG TCACCCATCA GGGCCTGAGC
CCTGTCGTTG ATGTGGAGT CGTCGACTCG TTTCGACTGAA TGCTCTTGT GTTTCAGATG CGGACGCTTC ACTGGTAGT CCCGGACTCG
193 D S T Y S L S S T L T L S K A D Y E K H K V Y A C E V T H Q G L S
^Constant Light cys

1101 TCGCCCGTCA CAAAGAGCTT CAAACAGGGAA GAGTTTAAAT TAAATCCTCT ACGCGGGAGC CATCGGGTAC CGGGGGATCT AGGCCTAACG
AGCGGGGAGT GTTGTCTCGAA GTTGTCCCT CTCACAATA ATTAGGAGA TGCGGCTCTGC GTGAGCACCGC TCGAGCCATG TCCGGATTCG
226 S P V T K S F N R G E C O
^cys to bind heavy

1201 CTCGGTTGCC GCGGGGGTT TTTCATTGTT GCGGACGGC ATCTCCAAATG AACTGTGTGC GCAGGTAGAA GCTTGGAGA TTATGTCAC TGCAATGCTT
GAGGCCAACGG C3GCCCGCAAA AAATAACAA CGGCTGCGCG TAGAGCTTAC TTGACACAGC CGTCCATCTT CGAAACCTCT AATAGCAGTG ACGGTACGAA
^end 1 lambda to 0 terminator

1301 CGCAATATGG CGCAAAATGAA CCAAACAGGG TTGATTGATC AGGTAGAGGG GGCAGCTGTAC CAGGTAAAGC CCGATGCCAG CATTCTGAC GACGATAACGG
GGGTATACCG GCGTTTACT GGTGTGCGCC AACTAACTAG TCCATCTCCC CGCGACATG CTCCATTTCG GGCTACGGTC GTAAAGGACTG CTGCTATGCC

1401 AGCTGCTGG CGATTAGTAA AGGAAGTT TGAAGCATCC TCGTGTGAA AAAGTTAATC TTTCAAACAG CTGTCAATAA GTTGTCACGG CGAGACTTA
TCGACGAGC GCTAATGCAAT TTCTCAATA ACTTCGTTGG AGCAGTCATT TTCAATTAG AAAGTTGTC GACAGTATT CRACAGTGC GGCTCTGAA

1501 TAGTCGCTT GTTTTATT TTAAATGTT TTGTAACTAG TAGGCAAGTT CAGCTAAAAA CGGTATCTAG AATTATGAG AAGATAATCG CATTTCCTCT
ATCAGGGAA CAAATAAA AAATACTAA AACATTGATC ATGCGTCAA GTGCATTTCG CCGATTTT CCCATAGATC TTAATACRTC TTCTATAGC GTAAAGAGAA
1 M K K N I A F L L
^STII Signal TIR-1

1601 TGCATCTATG TTTCGTTTT CTATTCGTCAC AAACGCGTAC GCTCAAGCTTC AGCTGGTCA GCTTCGGGCA GAGGTGAAA AGCZAGGGC TTCAGTAAA
ACGTAGATAC AAGCAAAAA GATAACGATG TTTCGCGATG CGAGTCGAGT CAGACCGCT CTCCACTTT TCGTCCCGG AGTCAAATT
10 A S M F V F S I A T N A Y A Q V L V Q S G A E V K K P G A S V K
^anti-VEGF Heavy Chain (VNERK version)

1701 GTATCCGTAA AGCTCTGG CTATACCTTC ACCAAACTATG GTATAAAACTG GGTCAGCTTC AGCTGGTCA GGTTCGGTCAG GCCCCGGGTA AGGGCTGGA ATGGTTGGA TGGATTAACCA
CATAGGACAT TTGAGAACCC GATATGAAAG TGGTGTATAC CATATTGAC CCAGGGAGTC CGGGGGCCAT TCCGGGACCT TACCCAACCT ACCTAATTCT
43 V S C K A S G Y T F T N Y G I N W V R Q A P G K G L E W V G W I N T
^Variable Heavy (VH) cys

1801 CCTATACCGG TGAACGACC TATGCTGG ATTCAAACG TCGTTTACT TTTCCTTAG ACACCTCCA AAGCACAGCA TACCTGCAGA TGAACAGCCT
GGATATGCC ACTGGCTGG ATACGACGCC TAAAGTTGCA AAAAGAATGTA ATGAGCTGT ATGGAGCTCT ACTTGTGCGA
77 Y T G E P T Y A A D F K R R F T F S L D T S K S T A Y L Q M N S L

FIG. 17C

Inventor: SIMMONS
Docket No.: 11669.120USU1
Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING
YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE
Attorney Name: KATHERINE M. KOWALCHYK
Phone No.: 612.371.5311
Sheet 25 of 50

11901 GCGGCGCTGAG GACACTGCG CGCTTACTG TGCAAAAGTAC CGGCACTATT ATGTAACAGA GCGGAAGAGC CACTGGATT TCGACGTCTG GGCTCAAGGA
 11902 CGCGGCGACTC CTGCTAAGGGC AGATAATGAC ACGTTTCTG GGCCTCTCTG GTGACCTATAA AGCTGAGAC CCAGCTTCTCTG
 110 R A E D T A V Y Y C A K Y P H Y Y V N E R K S H W Y F D V W G Q G
 ^vh cys

20001 ACCCTGGTCA CGGTCTCCTC GGCGCTCCACC AAGGGCCCAT CGGTCTTCCC CCGGACCCCC TCCCTCAAGA GCACCTCTGG GGGCACAGCG
 143 T L V T V S S A S T K G P S V F P L A P S S K S T S G G T A A L G C
 CGGACCGAGT CCTGATGAG GGCAGAGGG CGCGAGCTGG TTCCCGGGTA GCGAGAAGGG AGGAGGTCTC CGTGGAGAAC CCCGGTGTGC CGGACCCGA
 177 L V K D Y F P E P V T V S W N S G A L T S G V H T F P A V L Q S S
 CGGACCGAGT CCTGATGAG GGCAGAGGG CGCGAGCTGG TTCCCGGGTA GCGAGAAGGG AGGAGGTCTC CGTGGAGAAC CCCGGTGTGC CGGACCCGA
 22001 AGGACTCTAC TCCCCTAGCA GCGGTGGTAC TGTCGCCCCCTG AGCAGCTTGG GCGACCCAGAC CTAGATCTGC AACGTTGAAATC ACAAGCCCCAG CAACACAA
 210 G L Y S L S S V V T V P S S L G T Q T Y I C N V N H K P S N T K
 TCCCTGAGATG AGGGACTCGT CGCACCAGT ACACGGGAGA TGTCGAACCG AGGACTCTAC TCCCCTAGCA GCGGTGGTAC TGTCGCCCCCTG GTTGTGGTTC
 ^vh cys

23001 GTGGACAGA AAGTGGGCC CAATCTCTG GACAAACTC ACACATGCC ACCGTGCCCA GCACCTGAAAC TCCCTGGGG ACCGTCAAGTC TTCCCTCTCC
 243 V D K K V E P K S C D K T H T C P P C P A P E L L G G P S V F L F P
 CACCTGTTCT TTCAACTCGG GTTGTAGCA CTGTTGAG TGTGTACGG TGTCAGGGT CGGACCTGGT AGGAGGAAGG CGTGGACTTGG AGGACCCCC
 ^cys to bind light chain ^hinge cys

24001 CCCCAAACCC AAGGAGACCC CTCATGACTT CCCGGACCCC TGAGGTACA TGGGTGTTGG TGGGCTGTGAG CCACGAGAC CCTGAGGTCA AGTTCAACTG
 277 P K P K D T L M I S R T P E V T C V V D V S H E D P E V K F N W
 GGGGTTGG GTTCCTGTGG GAGTACTAGA GGGCTGGG ACTCTAGTGT ACCGACCCAC CGTGGACTTGG AGGAGGAAGG CGTGGACTTGG AGGACCCCC
 ^cys to bind light chain ^hinge cys

25001 GTACGGTGCAC GGGGGGGAGG TGGCTATAATGCC CAAGACAAAG CGGGGGAGG AGGACTACAA CAGCACGTAC CGTGTGGTCA GCGTCTCTAC CGTGTGGTCA
 310 Y V D G V E V H N A K T K P R E E Q Y N S T Y R V V S V L T V L H
 CATGCACTG CGGACCTTC AGTAAATTAG GTTCTGTTC GGGGCCTCC TCGTGTAGTT GTGTGTAGTG GCACACAGT CGCAGGAGTG CGGAGACGTG
 ^vh cys

26001 CAGGACTCTGC TGAAATGGCA GAGTACAG TGCAAGGTCT CGAACAAAGC CCTCCAGGC CCCATGAGA AAACCATCTC CAAAGCCAAA GGGCAGCCCC
 343 Q D W L N G K E Y K C K V S N K A L P A P I E K T I S K A K G Q P R
 GTCCCTGACCC ACTAACGGT CTCATGTTTACGGTCTG GGTTGTTCTG GGTTGAGTTCTT TTGGTAGAG GTTGTGGTCTT CCCTGTCGGGG
 ^cys

27001 GAGAACCCACA GTGTACACC CGTGCCTCCAT CCCGGCCAT GATGACCAAG AACCAGTCA GGCTGACCTG CCTGGTCAA GGCTGACTATC CCAGCGACAT
 377 E P Q V Y T L P P S R E E M T K N Q V S L T C L V K G F Y P S D I
 CTCTGGTGT CCACATGTGG GACGGGGTAA GGGCCCTCTC CTACTGGTC TTGGTCCAGT CGGACTGAGC GGACCAATTI CGGAAGATAG GTTGTGGTGA
 ^cys

FIG. 17D

Inventor: SIMMONS
Docket No.: 11669.120USU1
Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING
YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE
Attorney Name: KATHERINE M. KOWALCHYK
Phone No.: 612.371.5311
Sheet 26 of 50

2801 CGCCGCTGGAG TGGGAGGAGCA ATGGGGAGGCC GGAGAACAAAC TACAAGACCA CGGCCCTCCCGT GACGGGACTCC GCTGGCTCTCTA CAGGAAGGCTC
410 GCGGGACCTTC ACCCCTCTCGT TACCCGTCTGG CCTCTTGTGGT ATGTTCTGGT GCGGGAGGA AGAAGGAGAT GTGGTTGAG
A V E W E S N G Q P E N N Y K T T P P V L D S D G S F F L Y S K L
2901 ACCGTGAGCA AGAGGAGGTG GCAGCGAGGG AACGTCCTCT CAGTCCTCGT GATGCTGAG GCTCTGACCA ACCACTACAC GCAGAAAGAGC CTCTCCCTGT
443 T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L S L S
GGCACCTGT TCTCGTCCAC CGTCTGTCAC CGTCTGTCAC CGTCTGTCAC CGTCTGTCAC CGTCTGTCAC CGTCTGTCAC CGTCTGTCAC CGTCTGTCAC
^CH3 CYS
3001 CTCCGGCTAA ATAAGGATGC GACGGCCCTAA GAGTCCTAA CGCTCGGTTG CCGCCGGCG TTTCATTG TTAACTCATG TTTCACAGCT TATCATCGAT
477 P G K O
GAGGCCATT TATTGGTAGC CTGCGGGAT CTCAGGGAT GCGAGCAAC GGGCCCGC AAAAAATRAC AATTGAGTAC AAACTGTCGA ATAGTAGCTA
^start lambda t0 terminator ^end lambda to terminator ^start of tet resistance promoter
^start of tet resistance promoter ^-35 of promoter
3101 AAGCTTAAAT GCGGTAGTT ATCACAGTTA ATTGCTAAC GGAGTCAGGC ACCGTGATG AAATCTAAC ATGGCTCAT CGTCATCCTC GGCACCGTCA
TTCGAAATT CGCCATCAA TAGTGTCAAT TTAACGATTG CGTCAGTCCG TGGCATAC TTAGATGT TAGGCGAGTA GCAGTAGGAG CGGTGGCAGT
^ -10 region of tet resistance promoter ^-10 region of tet resistance promoter
^start of tet resistance translation
3201 CCCCTGGATGC TGTAGGCATA GGCTTGGGTA TGCGGGTACT GCCGGGCCTC TGCGGGATA TCGTCCATTIC CGACAGGATC GCCAGTCACT ATGGGTGCT
GGGACCTTAGC ACATCCGTAT CGAACCAAT AGGGCCATGA CGGGCGAG AACGGCCTAT AGGAGGTAAAG GCTGTCTGAG CGGTAGTGA TACCGCACGA

FIG. 18A

Inventor: SIMMONS
Docket No.: 11669.120USU1
Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING
YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE
Attorney Name: KATHERINE M. KOWALCHYK
Phone No.: 612.371.5311
Sheet 27 of 50

1 GAATTCACT TCTCCCTACT TTGATAAGG AATACAGAC ATGAAAATC TCATTCTGA GTGTATT AAGCTTGC
CTTAAGTGA AGAGGTGA AACATTCCTT TTATGCTG TACTTTAG AGTAAGACT CAACATAAA TTGAAACGGG
101 GAACCTGTG CGCAGGTAGA AGCTTGGAG ATTATCGTC CTGCAATGCT TCGGATATG GCGCAAATG ACCAACAGGG
CTTGACACAC GCGTCCATCT TCGAAACCTC TAATAGCTG AGCTTGAAG ACCGAAATG TGTTGTCGC CAACTAACTA
201 GGGCGTGTG CGAGGTAAAG CCCGATGCCA GCATTCCTGA CGACGATAG GAGCTCTGC GCGATTACGT AAAGAAAGTA
CCCGCACT GCTCCATTG GGGCTACGT GTAAAGGACT GTAAAGGACT CTCGATGCTGC CGCTAATGCA TTCTCTCAAT
301 AAAAGTTAAAT CTTTCAACA GCTGTCATAA AGTTGTCAGG GCGGAGACTT ATAGTCGCTT TGTTTTATT TTTAAATGTA
TTTCAATTA GAAAGTGT CGACAGTATT TCAACAGTGC CGGCTCTGAA TATZGCGAA ACAAAATAA AAAATTAAT
401 TCACGTAAA AGGGTATCTA GAATTGAA GAAGAAATAC GCATTTCTC TIGATCTAT GTTCGTTTT TCTATIGCTA
AGTCGATTT TCCCATAGAT CTTAAATCT CTTAACTATAG CGTAAAGGAG AACGTAGATA CAAGCAAAAA AGATAACGAT
1 M K K N I A F L L A S M F V F S I A T N A Y A D I
^STII signal TIR -1 ^variable light (VL) cys
501 CAGTCACCC AGTCCCCAG CTCCTCTGTC GCCTCTGTGG GCGATAGGG CACCATCACC TGGAGCGAA GTCAAGGATAT
GTCAACTGGG TCAGGGGTC GAGGGACAGG CGGAGACACC CGCTATCCCA GTGGTAGTGG ACGTGCGCTT CAGTCCTATA
26 Q L T Q S P S S L S A S V G D R V T I T C S A S Q D I S N Y L N W Y
^variable light (VL) cys
601 ATCAACAGAA ACCAGAAAA GCTCCGAAG TACTGATTA CTTCACCTCC TCTCTCCACT CTGGACTCCC TTCTCGCTTC
TAGTGTCTT TGGTCTCTT CGAGGCTTC ATGACTAAAT GAAGTGGGG AGAGAGGTGA GACCTCAGGG
60 Q Q K P G K A P K V L I Y F T S S L H S G V P S R F S G S G T
701 GGATTCACT CTGACCATCA GCAGTCTGCA GCCAGAAGAC TTGCAACTT ATTACTGTC ACAGTATGCA ACCGTGCGGT
CCTAAAGTGA GACTGGTAGT CGTCAGACGT CGGTCTCTG AAGCCTGAA TAATGACAGT TGTCATATCG TGGCACGGCA
93 D F T L T I S S L Q P E D F A T Y Y C Q Q Y S T V P W T F G Q G T
^variable light cys
801 AAGGTGAGA TCAAACGAAC TGTGGCTGCA CCATCTGCT TCATCTTCCC GCCATCTGAT GAGCAGTGA
TTCCACCTCT AGTTGCTTG ACACCGACGT GGTAGACAGA ACTAGAAGGG CGGTAGACTA CTGTCAACT TTGACCTTG
126 K V E I K R T V A A P S V F I F P P S D E Q L K S G T A S V V C L L
^variable light cys
Constant Light cys^

FIG. 18B

Inventor: SIMMONS
Docket No.: 11669.120USU1
Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING
YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE
Attorney Name: KATHERINE M. KOWALCHYK
Phone No.: 612.371.5311
Sheet 28 of 50

901 TGATAACTT CTATCCAGA GAGGCCAAG TACAGTGGAA GGTGGATAAC GCCCTCCAAT CGGGTAACTC CCAGGAGGT GTCACAGAGC AGGACAGCAA
ACTATGAA GATAGGTCT CTCGGTTTC ATGTCACCTT CCACCTATTT CGGGAGGTTA GCGGATTTGAG GGTCCCTCTCA CAGTGTCTCG TCCTGTCGTT
160 N N F Y P R E A K V Q W K V D N A L Q S G N S Q E S V T E Q D S K

1001 GGACAGCACC 'TACAGCTCA GCAAGCACCCT GAGCGCTGAGC AAAGCAGACT AGCAGAACAA CAAGCTAC GCCTGCGAG AGTCACCTACA GGGCTGAGC
CCCTGCGTGG ATGTCGGAGT CGTCGGAGA CTGCGACTCG TTTCGTCGA TGCTCTTGT GTTCAGATG CGGACGCTTC AGTGGTAGT CCCGGACTCG
193 D S T Y S L S S T L T L S K A D Y E K H K V Y A C E V T H Q G L S
^Constant Light cys

1101 TCGCCGCTCA CAAAGGCTT CAAACAGGGAA GAGTGTAAAT TAAATCCTCT ACCCGGAGC CATCGTGCG AGCTCGGTAC CGGGGGATCT AGGCTTAACG
AGCGGCGAGT GTTTCTCGAA GTTGTCCCTT CTCACAAATTA ATTAGGAGA TGCGGCTGC GTAGCACCGC TCAGGCCATG GCCCCCTAGA TCCGGATTGCG
226 S P V T K S F N R G E C O
^cys to bind heavy

1201 CTCGGTGGCC GCCGGCGTT TTTATTTGT GCGGACGCGC ATCTCGAATG AACTCTGTGC GCAGGTGAGA GCTTGTGAGA TTATCGTCAC TGAATGCTT
GAGCCGAGG CGGGCCGCA AAAATAACCA CGGCTGCGGC TAGAGCTAC TTGACACACG CGTCCATCTT CGAAACCTCT AATAGCAGTG ACGTTACGAA
^end lambda r0 terminator

1301 CGCAATATGG CGCAAAATGA CCAACAGCGG TTGATTGATC AGGTAGAGGG GGGGGTGTAC GAGGTAAAGC CGGATGCCAG CATTCCGTAC GACGATAACGG
GGGTATAACC GCGTTTACT GGTGTCGCC AACTAACTAG TCCATCTCCC CCGCACATG CTCCATTTC GGCTACGGTC GTAAGGACTG CTGCTATGCC

1401 AGCTGCTGG CGATTACGTA AAGAAGTTAT TGAAGCATCC TCGTCAGTAA AAAGTTAATC TTTCACAG CTTTCACAG CTTGTCAAA GTTGTCACTGG CGGAGACTTA
TCGACGAGGC GCTAATGCTT TTCTTCATA ACTTCGTTAG AGCAGTCATT TTTCATTAG AAAAGTTCCT GACAGTATT CAACAGTGCC GGCTCTGAAT

1501 TAGTCGCTTT GTTTTATT TTTAATGTT TTGTAACTAG TAGGAAAGT CACGTAAAAA GGTTATCTAG ATTATGAG AAGAATATCG CATTCTCT
ATCAGGGAAA CAAAATAAA AAATTACATA AACATTGATC ATGGTCTCAA GTGCAATTTC CCACTAGATC TTAATCTCTAGC GTAAAGARGA
1 ^Heavy chain FRI changed to Subgroup II consensus sequence

1601 TGCATCTATG TTGGTTTTT CTATTCGCTAC AACAGCGTAC GCTCAGGGTT AGCTGCAAGA GTCTGGCCCG GGCCTGGTGA AACATCTCA GACTCTCTCC
ACGTAGATAC AAGCAAAA GATAACGATG TTGTCGCTAT CGACTCCAG TCGACCTCT CCGGACCACT TTGGTAGAGT CTGGAGAGG
10 A S M F V F S I A T N A Y A Q V L Q E S G P G L V K P S Q T L S
^anti-VEGF heavy chain (VNFRK version)

1701 TTGACTCTGA CTGTTCTGG CTATACCTTC ACCAACTATG GTATAAACTG GGTCCCTCAG GCCTGGGTTA AGGCCTGGA ATGGGTGGA TGGATTAACA
AACTGAACAT GACAAGACC GATATGGAG AGTGGTAGATC CATATTGAC CCAGGCACTC CGGGGCCAT TCCCAACCT ACCTAAATTGT
43 L T C T V S G Y T F T N Y G I N W V R Q A P G K G L E W V G W I N T
^Variable Heavy (VH) cys

1801 CCTATACCGG TGAACCGACC TATGCTGCGG ATTCAAAAGC TCGTTTCAACT TTTTCTTAG ACACCTCAA AAGCACAGCA TACCTGAGA TGAACAGCCT
GGATATGGCC ACTTGGCTGG ATAGACGCC TAAAGTTGC AGCAGAAGTAAAGAATC TGTTGGAGGT TICGTTGTCGT ATGGACGCT ACTTGTGGGA
77 V T G E P T Y A A D F K R R F T F S L D T S K S T A Y L Q M N S L

FIG. 18C

1901 GCGCCCTGAG GACACTCCG TCTATTACTG TGCAAAGTAC CGGCACTATT ATGTAACGA GCGGAAGAGC CACTGGTATT TCGACGTCTG GGGTCAAGGA
CGCGCGACTC CTGTGACGGC AGATAATGAC AGTGTTCATG GGCCTGATAA TACACTTGT CGGCTTCTCG CCCAGTTCCCT
110 R A E D T A V Y Y C A K Y P H Y V N E R K S H W Y F D V W G Q G
^VH cys

2001 ACCCTGGTCA CGCTCTCTC GGCTCCAC AAGGGCCAT CGGTCTTCCC CCTGGCACCC TCCCTCAAGA GCACCTCTGG GGGCACAGGG GCCCTGGGCT
TGGGACCGT GGCGAGGGAG CGGGAGGTGG TCCCGGGTAA GCGAGAAGGG GGACCGTGGG AGGAGGTCTT CGTGGAGACCC CGGGTGTGCG CGGGACCCGA
143 T L V T V S S A S T K G P S V F P L A P S S K S T S G G T A A L G C
CGGACCAAGTT CCTGATGAG GGGCTGGCC ACTGCAACAG CACCTGTAGT CGCGGGACT GGTGGCGCA CGTGTGGAG GGCGACAGG ATGTCAGGAG
177 L V K D Y F P E P V T V S W N S G A L T S G V H T F P A V L Q S S

2101 GCGCTGTCAA GGACTACTTC CCCGAACCCG TGCGGAACCTCA GCGCGCCTGA CCAGCCCGGT GCACACCTTC CGCGCTGTCC TACAGTCCTC
CGGACCAAGTT CCTGATGAG GGGCTGGCC ACTGCAACAG CACCTGTAGT CGCGGGACT GGTGGCGCA CGTGTGGAG GGCGACAGG ATGTCAGGAG
177 L V K D Y F P E P V T V S W N S G A L T S G V H T F P A V L Q S S

2201 AGGACTCTAC TCCCTCTAGCA GCGTGGTGCAC TGTGCGCTCT AGCAGCTTGG GCACCCAGAC CTACATCTGC AACGTGAATC ACAAGCCCCAG CAACACCAAG
TCCCTGAGATG AGGGACTCTG CGCAACCACTG ACACGGGAGA TCGTCGAACC CGTGGCTCTG GATGTAGACG TTGCACTTAG TGTCGGGTC GTGGTGGTTC
210 G L Y S L S S V V T V P S S L G T Q T Y I C N V N H K P S N T K
^CH1 cys

2301 GTGGACAAAGA AAGTTAGGCC CAAATCTGT GACAAAACT ACACATGGCC ACCGTTGCCA GCACCTGAAC TCCCTGGGG ACCGTCAGTC TICCTCTTCC
CACCTGTTCT TTCAACTCGG GTTGTAGACA CTGTGTTGAG TGTGTAACGGG TGGCAAGGGT CGTGGACTTG AGGACCCCCC TGGCAGTCAG AAGGAGAAGG
243 V D K K V E P K S C D K T H T C P P C P A P E L L G G P S V F L F P
^cys to bind light chain ^hinge cys

2401 CCCCTAAACCC CAAAGGAGCC CTCAGTATCT CCCGGCCCC TGAGGTACAA TCGCTGGTGG TGACACGTGAG CCACGAGAGC CCTGAGGTCA AGTTCAACTG
CACCTGTTGG GTTCTCTGG GAGTACTAGA GGGCTGGGG ACTCCAGTGT ACGCACCCACC ACCTGCACT GCCTGCTTCTG GGACTCCAGT TCAAGTTGAC
277 P K P K D T L M I S R T P E V T C V V D V S H E D P E V K F N W
^cys to bind light chain ^hinge cys

2501 GTACCTGGAC GGGGTGGAGG TGCTATATGC CAAGACAAG CGCGGGAGG ACCAGTACAA CAGCACGTAC CGTGTGGTCA CGCTCTGCAC CGTCTCTGCAC
CATGCACTG CGCACTCTC ACGTATACG GTTCTGTTTC GGCGCCCTCC TCGTCTATGT GTCTGCTCATG GCACACAGT CGCAGGAGTG GCAGGACGCTG
310 Y V D G V E V H N A K T K P R E E Q Y N S T Y R V V S V L T V L H

2601 CAGGACTGGC TGAATGGCAA GGAGTAGAG TGCAAGGGTCT CCAACAAAGC CCTCCAGCC CCCATCGAGA AAACCAATCTC CAAAGCCAAA GGGCAGCCCC
GTCCCTGACCG ACTTACCGTT CCTACATCTC ACGTTCAGA GTTGTGGTGG GGAGGTGGTCT TTGGTAGCTT GTTGGTAGAG GTTTCGGTTT CCCGTGGGG
343 Q D W L N G K E Y K C K V S N K A L P A P I E K T I S K A K G Q P R
^CH2 cys

2701 GAGAACCA GGTGTACACC CTGGCCCCAT CCCGGAAAGA GATGACCAAG AACAGGTCA GCTGGTACCTG CCTGGTCAA GGTGTCTATC CCAGGACAT
CTCTTGTTGT CCACATGGG GACGGGGTAA GGGCCCTCT CTACTGGTCT TTGGTCCAGT CGGACTGGAC GGACCACTTT CGGAAGATAG GTTCGGCTGTA
377 E P Q V Y T L P P S R E E M T K N Q V S L T C L V K G F Y P S D I
^Constant Heavy 3 (CH3) cys

FIG. 18D

Inventor: SIMMONS
Docket No.: 11669.120USU1
Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING
YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE
Attorney Name: KATHERINE M. KOWALCHYK
Phone No.: 612.371.5311
Sheet 30 of 50

2801 CGCCCTGGAG TGGGAGAGCA ATGGGCAAGCC GGAGAACAAAC TACAAGACCA CGGCTCCCGT GCTGGACTCC GACGGCTCTT TCTTCCCTTA CAGGAGAGCT
410 GCGGCAACCTC ACCCTCTCGT TACCCGCTCG CTCCTCTGGT ATGTTCTGGT CGCCAGGGCA CGACCTGAGG CTGCCGAGGA AGAAGGAGAT GTCGTTGAG
2901 ACCGTCGACA AGAGCCGGTG GCAGGCAAGGG AACGTCCTCT CATGCTCCGT GATGCTGAG GCCTCTGACA ACCACTAC GCAGAAAGAGC CTCTCCCTGT
443 T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L S L S
477 P G K O ^start lambda to terminator ^end lambda to terminator ^start of tet resistance promoter
^end of tet resistance promoter
3101 AAGCTTAAAT GCGGAGTTT ATCACAGTTA ATTGCTAAC GCAGTCAGGC ACCGTTATG AAATCTAAC ATGCTAACAT CGTCATCCTC GGCACCGTCA
TTGGAAATA CGCCATCAA TAGTGTCAAT TAACTGTTG CGTCAGTCCG TGGCATACTA TTAGATGT TACGGAGATA GCAGTAGGAG CCGTGGCAGT
-10 region of tet resistance promoter ^start of tet resistance translation
3201 CCCCTGGATGC TGTAGGGATA GGCTTGGGTA TGCCGGCTAC GCCGGGCTTC TGGGGATA TCGTCCATTIC CGACAGGATC GCCAGTCACT ATGGGTGCT
GGGACCTTAGC ACATCCGTAT CGAACCAAT AGGCCATGA CGGCCATGA AACCCGGAG AGCAGGTAAG GCTGTCGAG CGTCAGTGA TACGGACGGA

FIG. 19A

Inventor: SIMMONS
Docket No.: 11669.120USU1
Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING
YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE
Attorney Name: KATHERINE M. KOWALCHYK
Phone No.: 612.371.5311
Sheet 31 of 50

1 GAATTCACT TCTCCATACT TTGATAAGG AAATACAGAC ATGAAAATC TCATTGCTGA GTTGTATT AAGCTTGCCC AAAAGAAGA AGAGTCGAAT
CTTAAGTGA AGAGGTATGA AACTATTC TTATGCTG TACTTTAG AGTAAAGACT CAACAAATAA TTGAAACGGG TTTTCTCT TCTAGCTTA
101 GAACTGCTG CGCAGGTTAGA AGCTTGGAG ATTATCGCTA CTGCAATGCT TCGAAATATG GGCAAAATG ACCAACAGGG GTTGATGAT CAGGTAGGG
CTTGACACAC GCGTCACATCT CGGAAACCTC TAATAGCAGT GACGTAGA AGCCTTAC CGCTTAC CGCTTAC CAACTAACTA GTCCATCTCC
201 GGGCGCTGTA CGAGGTAAG CCCGATGCCA GCGATGCCA GCATTCCTGA CGACGATACG GAGCTGCTGC GGATTAAGT AAAGAAAGTA TTGAAGCATC CTGTCAGTA
CCCGCAGAT GCTCCATTG GGGCTACGGT CGTAAAGGACT GTTAAAGGACT CTGCCTATGC CTGCCTATGC CGCTAAATSCA TTTCCTCAT AACTTGTTAG GAGCAAGTCA
301 AAAAGTATTAT CTTTCACAA GCTGTCATAA AGTTGTCACG GCGGAGACTT ATAGTCGCTT TTGTTTATT TTAAATGTA TTGTAACTA GTAGCAAGT
TTTCATTA GAAAAGTGT CGACAGTATT TCAACAGTGC CGGCTCTGAA TATCAGCAGA ACAAAATAA AAAATTACAT AAACATGAT CATCGTTCA
401 TCACGTAAA AGGGTATCTA GAATTATGAA GAGAATATC GCATTCTTC TTGCTATCTAT GTTGTGTTT TCTATGCTA CAAACGGTA CGCTGATATC
AGTGCATTTC TCCCATAGAT CTTAAACTT CTCTTATAG CGTAAAGAAG AACGTAGATA CAAGGAAAAA AGATAAACGT GTTGGCCAT GCGACTATAG
M K K N I A F L L A S M F V F S I A T N A Y A D I
1 ^STII signal TIR -1 Anti-VEGF light chain (version Y0317) ^

501 CAGTTGACCC AGTCCCCGAG CTCCCTGTCC GCGATAGGGT CACCATCACC TGAGGCCAA GTCAAGGCCAA
GTCAACTGG TCAGGGGCTC GAGGGACAGG CGGAGACAC CGTATCCCA GTGGTAGTGG ACGTGCGGTT CAGTCCTATA ATCGTTGATA AATTGACCA
26 Q L T Q S P S L S A S V G D R V T I T C S A S Q D I S N Y L N W Y
601 ATCAACAGAA ACCAGAAAA GCTCCGAAAG TACTGATTAA CTTCACCTCC TCTCTCCACT CTGGAGTCCC TTCTCGCTTC TCTGGATCCG GTTCTGGGAC
TAGTTGCTT TGGTCCTTC CGAGGCTTC ATGACTAAAT GAAGTGGAGG AGAGCTGTA GACCTCAGGG AAGAGCGAAG AGACCTAGGC CAAGACCCCTG
60 Q Q K P G K A P K V L I Y F T S S L H S G V P S R F S G S G S G T
701 GGATTTCACT CTGACCATCA GCAGTCGTCA GCGAGAAGAC TTGCAACTT ATTACTGTCAC AGACTATAGC ACCGTGCCG GTACGGTTGG ACAGGGTACCC
CCTAAAGTGA GACTGGTAGT CGTCAGACGT CGCTCTCTG AAGGCTTGA TAATGACAGT TGTCATATCG TGGCACGGCA CCTGCAAAAC TGTCCATGG
93 D F T L T I S S L Q P E D F A T Y Y C Q Q Y S T V P W T F G Q G T
801 AAGGGGAGA TCAAAGAAC TGTTGGCTGCA CCATCTGCT TCACTCTGAT GAGCAGTGA AATCTGGAAAC TGCTTCTGTT GTGTCCTG
TTCCACCTCT AGTTGCTTG ACACCGACGT GGTAGACAGA AGTAAAGGG CGGTAGACTA CTCGTCAACT TTAGACCTG ACGAAGACAA CACGGACG
126 K V E I K R T V A A P S V F I F P S D E Q L K S G T A S V V C L L
901 TGAATAACCT CTATCCAGA GAGGCCAAG TAGCTGGAA GGTTGGATAAC GCCCCTCCATT CGGGTAACIC CCAGGAGAGT GTCACAGAGC AGGAGCAGCAA
ACTTATGAA GATAGGGTCT CTCGGTTTC ATGTCACCTT CCACCTATT CGGGAGTTA GCCCATTGAG GGTCTCTCA CAGTGTCTCG TCCCTCTGTT
160 N N F Y P R E A K V Q W K V D N A L Q S G N S Q E S V T E Q D S K

FIG. 19B

Inventor: SIMMONS
Docket No.: 11669.120USUI
Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING
YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE
Attorney Name: KATHERINE M. KOWALCHYK
Phone No.: 612.371.5311
Sheet 32 of 50

1001 GGACAGCACC TACAGCTCA GCAGCACCT GCAGCTGAGC AAAGCAGACT ACAGAAACA CAAAGTCTAC GCCTGCGAAG TCACCCATCA GGGCCTGAGC
CCTGCTGCG ATGTGGAGT CGTCTGGGA CTGCTGGGA TTGCTGACTCG 193 D S T Y S L S T L S K A D Y E K H K V Y A C E V T H Q G L S

1101 TCGCCCTCA CAAAGAGCTT CAACAGGGGA GAGTGTAAAT TAAATCCTCT ACGGGGGAGC CATCGTGGCG AGCTCGGTAC CGGGGATCT AGGCCTAACG
AGCGGGGAGT GTTCTCGAA GTTGTCCCT CTCACAATTAA ATTAGGAGA TGCGGCTGC GTAGGCCCG TCAGGCCATG GGGCCCTAGA TCGGATTGC
226 S P V T K S F N R G E C O

1201 CTCGGTCCC GCCGGGGT TTATATTGTT GCCGAGCGGC ATCTCGATG AACTCTGTC GCAGGTAGAA GCAGGTAGAA TTATCGTCAC TCAATGCTT
GAGGCCAGG CGGGCCGAA AAAATAACAA CGGTGCGGG TAGAGCTAC TTGACACAGC CGTCCATCTT CGAAACCTCT AATAGCAGTG ACGTACGAA

1301 CGCAATATGG CGCAAAATGA CCAACAGCGG TTGATTGATC AGGTAGGG GGGGGTGTAC GAGGTAAGG CGATGCCAG CATTCCGTAC GACGATACGG
GGGTATAACC CGGTGTTACT GGTGTCGCC ACTAAGCTAG TCCATCTCC CCGCACATG CTCCATTFTCG GGTCACGGTC GTAGGACTG CTGCTATGCC

1401 AGCTGCTCG CGATTAAGCTA AAGAAGTTAT TGAGGATCTC TGTCAGTAA AAAGTTAATC TTTCACAG CTGTCATAAA GTGTCACGG CCGAGACTTA
TCGACGAGGC GCTAATGCTT TTCTTCATA ACTTCGTTAG AGCAGTCATT TTTCATTAG AAAAGTGTCTC GACAGTATT CAACAGTGCC GGCTCTGAAT

1501 TAGTCGCTT GTTTTATT TTAATGTT TTGTTAATCTAG TAGCAAGTT CACCTAAAAA GGGTATCTAG AATTATGAG AAGAATATCG CATTTCCTCT
ATCAGGAAA CAAATAAA AAAATACATA AACATTGATC ATGCGTTCAA GTGZATTTC CCCATAGATC TAAATCTC TTCTTATAGC GAAAGAAGA
1 ^STII Signal TIR-1

1601 TGCATCTATG TTGCTTTT CTATTCGCTAC AAACGGTAC GCTCAGGGTTC AGCTGGTCA GTCAGGTTAC GAGGTGAAAG AGCCAGGGCA ATGGGTGGA TCGGATTAACA
ACGTAGATAC AAGCAAAAAA GATAACGATG TTGCGCGATG 10 A S M F V F S I A T N A Y A Q V L V Q S G A E V K K P G A S V K
^Anti-VEGF (version Y0317) Heavy Chain
^Heavy Chain FR1 changed to Subgroup I consensus sequence

1701 GTATCTGTA AAGCTCTGG CTAGGACTTC ACGCACTACG GTATGAACTCG GTCAGGTCA GGGCCGGTA AGGGCCCTGGA TCGGATTAACA
CATAGGACAT TTGGAAGCC GATGCTGAG TGCGTGTATGC 43 V S C K A S G Y D F T H Y G M N W V R Q A P G K G L E W V G W I N T
CCATACGG TGAACCCACC TATGCTGGG ATTCAAACG TGTTTCACT TTTCCTTAG ACACCTCCA AAGCACAGCA TACCTGAGA TGAACAGCCT
GGATATGCC ACTTGCTGG ATAGGACGCC TAAAGTTGC ACCAAAGTGA AAAGAAATC TGCGGAGTT TTGTTGCTGT ATGGACGTCT ACTGTCGGA
77 Y T G E P T Y A A D F K R R F T F S L D T S K S T A Y L Q M N S L

1901 GCGCGCTGAG GACACTCCG TCTATTACTG TGCAAAAGTAC CCGTACTATT ATGGACGAG CCACCTGGTAT TTGACGGTCT GGGGTCAGG AACCTGGTC
CGCGCGACTC CTGTCAGGGC AGATAATGAC AGTGTTCATG GGCATGATAA TACCCGTCTT GGTGACCCATA AAGCTGAGA CCCCAGTTC TTGGACCA
110 R A E D T A V Y Y C A K Y P Y Y G T S H W Y F D V W G Q G T L V

2001 ACCGTCCTCT CGGCTCCAC CAAGGGCCA TTGGCTCTCC CCGCTGGCAC AGCACCTCTG GGGGACAGGC GGCCCTGGGC TGCCTGGTC
TGGCAGAGGA GCGGAGGTG TTTCGGGGT AGCCAGAAGG GGGACCGTGG GAGGAGGTG TCGTGGAGAC CCCCCGGTGTG CGGGGACCCG AGGACCCAGT
143 T V S S A S T K G P S V F P L A P S S K S T S G G T A A L G C L V

FIG. 19C

Inventor: SIMMONS
Docket No.: 11669.120USUI
Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING
YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE
Attorney Name: KATHERINE M. KOWALCHYK
Phone No.: 612.371.5311
Sheet 33 of 50

2101 AGGACTACTT CCCGAAACCG GTGACGGGTG CGTGGAACTC AGGGGCCCTG ACCAGGCCGG TGACACCCCT CCCGGCTGTC CTACAGCCCT CAGGACTCTA
TCCTGATGAA GGGGCTGGC CACTGCCACA TGACCTTGAAC TCCGGGGAC TGCTGGCC ACGTGTTGGAA GATTCAGGA GTCTCTGAGAT
177 D Y F P E P V T V S W N S G A L T S G V H T F P A V L Q S S G L Y

2201 CTCCCTAGC AGCGTGGTGA CTGGCCCTC TAGAGCTTG GGACCCAGA CCTACATCTG CAACGTGAAT CACAAGCCA GCAACACCAA GTGGGACAAG
GAGGGATGCG TCGACCACT GACACGGGAG ATCGTGAAC CGCTGGCT GGATGTAGAC GTGGACTTA GTGTCGGGT CGTGTGGTT CCACCTGTT
210 S L S S V V T V P S S L G T Q T Y I C N V N H K P S N T K V D K

2301 AAAGTTGAGC CCAAAATCTTG TGACAAAACT CACACATGCC CACCGTCCCC AGCACCTGAA CTTCITGGGG GACCGTCAGT CTTCCTCTTC CCCCAAAAC
TTTCAACTCG GGTTAGAAC ACTGTTTGA GTGTGTACGG GTGGACGG CGTGTGGACTT GAGGACCCC CTGGCAGTCA GAGGGAAAG GGGGGTTTG
243 K V E P K S C D K T H T C P P C P A P E L L G G P S V F L F P P K P

2401 CCAAGGAC CCTCATGATC TCCGGGACCC CTAGGGTACG ATGGGTGCGTGA GTGGACCTGA GGCACGGAAA CCCTGAGGTG AACGTGAACT GGTAACCTGGA
GGTTCCTGTG GGAGTACTAG AGGGCCTGGG GACTCCAGTAG AGGGCTGGTT GACTGACAC CACCTGACT CGTGCTTCT GGAACTCCAG TCAAGTGA CCATGACCT
277 K D T L M I S R T P E V T C V V V D V S H E D P E V K F N W Y V D

2501 CGGCGTGGAG GTGCAATAATG CCAAGACAAA GGGGGGGAG GAGGACTACA ACAGGACCPA CGGTGTGGTC AGGGTCCTCA CGTCCTGCA CCAGGACTGG
GGCGACCTC CAGTATTAC GGTCTGTGTT GGAGTACTAG AGGGCCTCTC CTGGCTGTGT TGTCGTGCAAT GGACACCCAG TCGAGGAGT GGAGGAGCTGT GGTCCTGACC
310 G V E V H N A K T K P R E E Q Y N S T Y R V V S V L T V L H Q D W

2601 CTGAATGCA AGGAGTACAA GTGCAAGGTC TCCACAAGG CGCTCCAGC CCCATCGAG AAAACCATCT CCAAAGCCAA AGGGCAGCCC CGAGAACAC
GACTTACGT TCCTCATGTT CAGTTCCAG AGGTGTGGTC GGGGGTAGCTC TTGGTGTAGA GGTTTCGGTT TCCGGTGGG GCTCTTGTTG
310 G N K E Y K C K V S N K A L P A P I E K T I S K A K G Q P R E P Q

2701 AGGTGTACAC CCTGCCCCCA TCCGGGAG AGATGACCA AAACCAAGTC AGCCTGACCT GCTGGTCAA AGGCTCTAT CCCAGGACAA TCGCCGTGGA
TCCACATGTC GGACGGGGT AGGGCCCTTC TGTACTGGT CTGGTCAAG TGCACTGAA CGGACCACTT TCCGAAGATA GGTCGCTGT AGGGGACCT
377 V Y T L P P S R E E M T K N Q V S L T C L V K G F Y P S D I A V E

2801 GTGGGAGGC AATGGGCAGC CGGAGAACAA CTACAAGAC ACGCCCTCCG TGCTGGACTC CGACGGCTCC TCTTCCTCT ACAGGAAAGT CACCGTGAC
CACCCCTCG TTACCGCTCG GGCCTCTGG GATGTTCTGG TGCGGAGGC ACGACCTGAG GTGCGCGAGG TGTCGTGCA GTGCACTG
410 W E S N G Q P E N N Y K T T P P V L D S D G S F F L Y S K L T V D

2901 AAGAGGAGGT GGAGCAGGG GAAGGTCTTC TCATGCTCG TGATGCTGAA GGCTCTGCA ACCACTACA CGCAGAAGAG CCTCTCCCTG TCTCCGGTA
TTCTCGTCCA CGTGTCTCC CGTGTGAGG AGTACGAGGC ACTACGTAAT CGGAGCTGT TTGGTGTATGT GCGTCTCTC GGAGAGGAC AGAGGCCAT
443 K S R W Q Q N V F S C S V M H E A L H N H Y T Q K S L S L S P G K

3001 AATAAGCTG CGACGGCCCT AGAGTCCCTA ACGETCGGTT GCCGCCGGC GTTTTTATT GTAACTCAT GTTGACAGC TTATCATCGA TAAGCTTAA
TTATTCTGTCG GCTGCGGGAA TCTCAGGGAT TGCGAGGAA CGGGGGCCG CAAAGAAATAA CAATTGAGTA CAAACTGTGCG AATAGTAGCT ATTGAAATT
477 O

3101 TGGGTAGTT TATCACAGTT AATTTGCTAA CGGAGTCAGG CACCGTGTAT GAAATCTAAC AATGGGCTCA TGGTCATCT CGGCACCGTC ACCCTGGATG
ACGCCATCAA ATAGTGTCAA TTAACTGATT GCCTGCTGTC CTTTAGATTG TTACGCGAGT AGCAAGTGA GCGCTGCGAG TGGGACCTAC
Start Tet Resistance Coding Sequence

FIG. 19D

3201 CTGTAAGGAT AGGCTTGGTT ATGCCGGTAC TGCCGGGCTT CTTGGGGATT ATCGTCCATT CGGACAGGAT CGCCAGTCAC TATGGCGTGC TGCTAGCGCT
GACATCCGTA TCCGAACCAA TACGGCCATG ACGGCCCTAA GAACGCCGGA GGGCTGTGCTA GCGGTCAAGTG ATACCGCAG ATACCGCAGC AGATCGCGA

FIG. 20A

Inventor: SIMMONS
Docket No.: 11669.120USU1
Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING
YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE
Attorney Name: KATHERINE M. KOWALCHYK
Phone No.: 612.371.5311
Sheet 35 of 50

1 GAATTCAACT TCTCCATACT TTGGATAAGG AAATAACAGAC ATGAAAATC TCATTGTGAA GTTGTAAATT AAGCTTGCCTT AAAAGAAGA AGAGTCGAAT
CTTAAGTGA AGAGGTATGA AACATTAATCC TTATGTCIG TACTTTTAG AGTAACGACT CAAACATAAA TTGGAACGGG TTTTCTCTCT TCTCACGTT
101 GAACTGTGCG CGCAGGTAGA AGCTTTGGAG ATTATCGTCA CTGCAATGCT TCGCAATATG GCGCAAAATG ACCAACAGCG GTTGTATGAT CAGGTAGAGG
CTTGACACAC GCGTCATCT TGAAACCTC TAATAGCAGT GACGTAGA AGCGTTATAC CGCGTTATAC CGCGTTTAC TGTTGTGCG CAACTAACTA GTCCATCTCC
201 GGGCGCTCTA CGAGGTAAAG CCCGATGCCA CGATTICCTGA CGACCATAGC GAGGTGCTGC GCAGATTAGT AAAGAAAGTA TGAAGGATC CTCGTCAGTA
CCCGGAGAT GCTCCATTTC GGTACGGT CGTAAGGACT GCTGCTATGC CGTACGACG CGTAAATGCA TTCTTCAAT AACCTGTAG GAGCACTCAT
301 AAAAGTTAT CTTTCAAACG GCTGTCTAA AGTGTCTACGCCGAGACTT ATAGTCGCTT TGTGTGTATT TTTAAATGTA TTGTGTAACTA GTACGAAAGT
TTTCAATTAA GAAAGTTGT CGACAGTATT TCAACAGTGC CGGCTCTGAA TATCAGGAA ACACAAATAA AAAATTACAT AAACATGAT CATTGCTTCA
401 TCACGTAAAGGTTATCTA GAAATTATGAA GAAGAATATGC GCAATTCTTC TTGATCTAT GTTGTATCTAT GTTCGTTTTT TCTATTGCTA CAAACGGTA CGTGTATTC
AGTGCAATT TCCCATAGAT CTTACATCTT CTCTCTTATAG CGTAAGGAAAG AACGTAGATA CAAAGAAAAA AGATAACGAT GTTGGCGAT GCGACTATAG
1 M K K N I A F L L A S M F V F S I A T N A Y A D I
^STII signal TIR-1 anti-IgE light chain
501 CAGCTGACCC AGTCCCCGAG CTCCTGTCC GCCTCTGTGG GCGATAGGGT CACCATCACCG TGGCGTGCCTA GTCAAGAGCTT CGATTAGAT GGTGATAGCT
GTCGACTGGG TCAGGGCTC GAGGGACAGC CGGTATGCCA CGGTAGTGG ACGGCACGGT CAGTCTCGCA GCTAATGCTA CCACATATCGA
26 Q L T Q S P S S L S A S V G D R V T I T C R A S Q S V D Y D G D S Y
601 ACATGAACCTG GTATCAACAG AAACAGGAA AAGCTCCGA ACTACTCATT TACGCCGGCT CGTACCTGGA GTCTGGAGTC CCTTCTCGCT TCTCTGATC
TGTACTGAC CATACTGTC TTGGTCCTT TTGAGGCTT TGATGACTAA ATGCCGGGA GTATGGACCT CAGACCTGAGAAGGGGA AGAGACCTAG
60 M N W Y Q Q K P G K A P K L L I Y A A S Y L E S G V P S R F S G S
701 CGGTTCTGGG ACGGATTCA CTCTGACCAT CAGCAGTCTG CAGCCGGAG ACTTCGCAAC TTATTACTGT CAGCAAAGTC ACAGGGATCC GTACACATT
GCCAAGACCC TGCTTAAGT GAGACTGGTA GTCTCGACG GTCTGGCTTC TGAAAGCTGTG AATAATGACA GTCTCTCTAG TGCTCTAGG CATTGCTAAA
93 G S G T D F T L T I S S L Q P E D F A T Y Y C Q Q S H E D P Y T F
801 GGACAGGGTA CCAAGGTGG AATCAAAAGGA ACTGTGGCTG CACCATCTGT CCTCATCTC CGGCCATCTG ATGAGGAGTT GAAATCTGGA ACTGCCCTCTG
CCTGTCCTAT GGTTCACCT CTAGTTGGCT TGACACCGAC GTGTAGAGA GAAGTAGACA TACTCGTCAA CTTTAGACCT TGACGGAGAC
126 G Q G T K V E I K R T V A P S V F I F P P S D E Q L K S G T A S V
901 TTGTGTGGCT GCTGAATAAC TTCTATCCCA GAGAGCCAA AGTACAGCTGG AAGGTGGATA AGGCCCTCCA ATGGGTAAC TCCCAAGGAGA GTGTCACAGA
AACACAGGA CGACTTATGG AAGATAGGGT CTCTCGGGT TCATGTGACC TTCCACCAT TGCGGGAGGT AGGGTCCTCTG AGGGTCCTCTG
160 V C L L N N F Y P R E A K V W K V D N A L Q S G N S Q E S V T E

FIG. 20B

FIG. 20C

Inventor: SIMMONS
Docket No.: 11669.120USUI
Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING
YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE
Attorney Name: KATHERINE M. KOWALCHYK
Phone No.: 612.371.5311
Sheet 37 of 50

2101 TGGTCAAGGA CTACTCCCC GAAACGGGTGA CGGTGTCGTG GAACTCGGGC GCCCTGACCA GCGGGGTGCA CACCTCCCG GCTCTCTAC AGTCCTCAGG
ACCACTTCT GATGAAGGGG CTTGGCCACT GGCACAGCAC CTTGGTCC CGGGACTCT CGGGACTCT CGGGACTCT CGGGACTCT CGGGACTCT CGGGACTCT
173 V K D Y F P E P V T V S W N S G A L T S G V H T F P A V L Q S S G

2201 ACTCTACTCC CTCAGCAGCG TGGTGAATGT GCCCTCTAGC AGCTTGGCA CCCAGACCTA CATCTGAAAC GTGAATACA AGCCAGCAA CACCAAGGTG
TGAGATGAGG GAGTCGTCG ACCACTGACA CGGGAGATCG TCGAACCGT GGGTCGGAAT GTAGACGTT CACTTAGTGT TCGGGTGT GTGGTCCAC
206 L Y S L S V V T V P S S S L G T Q T Y I C N V N H K P S N T K V

2301 GACAAGAAG TTGAGCCCAA ATCTTGTGAC AAAACTCACA CATGCCAAC GTGCCAGCA CCTGAACTCC TGGGGGACCC GTCAAGTCTTC CTCCTCCCC
CTGTTCTTC AACTCGGGT TAGAACACTG TTGTGAGTGT GTACGTTGG CACGGTGTG GAACTTGAGG ACCCCCTTGG CAGTCAGAAG GAGAAGGGG
239 D K K V E P K S C D K T H T C P P C P A P E L L G G P S V F L F P P

2401 CAAACCCAA GGACACCCCTC ATGGATCTCCC GGACCCCTGA GGTCACTATGC GTGGTGGTGG ACGTGAGCCA CGAAGACCTT GAGGTOAATG TCAACTGTA
GTTTGGGT CCGTGGGGAGG TACTAGAGGG CCTGGGGACT CCAGTGTAGC CACCAACCC TGACTCTGGT GTCTCTGGGA CTTCAGTTCA AGTGTACCAT
273 K P K D T L M I S R T P E V T C V V D V S H E D P E V K F N W Y

2501 CGTGGACGGC GTGGAGGTGC ATATGCCAA GACAAAGGCC CGGGAGGAGC AGTACACAG CACGTACCGT GTGGTCAGGG TCTCTAACCGT CCTGACCCAG
GCACCTGGCG CACCTCCACG TATTACGGTT CTGTTTGGC GCCCTCTCG TCATGTTGTC GTCACTGGCA CACCACTGGCA AGGAGTGGCA GGACCTGGTC
306 V D G V E V H N A K T K P R E E Q Y N S T Y R V V S V L T V L H Q

2601 GACTGGTGA ATGGCAAGGA GTACAAGTGC AAGGTCTCCA ACAAGCCCT CCCAGCCCCC ATCGAGAAA CCATCTCCAA AGCCAAAGGG CAGGGCGAG
CTGACCCGACT TACCGTCTCT CATTGTCAG TTCCAGAGGT TGTTTGGGA GGGTGGGG TAGCTCTTT GTAGAGGTT TCGGTTCTCC GTGGGGCTC
339 D W L N G K E Y K C K V S N K A L P A P I E K T I S K A K G Q P R E

2701 AACCAACGGT GTACACCCCTG CCCCATCCC GGGAAAGAGAT GACCAAGAAC CAGGTCAAGC TGACCTGGCT GGTCAAAGGC TCTTATCCA GCGACATCGC
TTGGTGTCCA CATGGGGAC GGGGTAGGG CCTTCTCTA CTGGTTCTTG GTGGTTCTCG ACTGGACGGA CCAGTTCTCG AAGTATGGGT CGCTGTAGCG
373 P Q V Y T L P P S R E E M T K N Q V S L T C L V K G F Y P S D I A

2801 CGTGGAGTG GAGGCAATG GGAACAACTAC AAGACCAAGC CTCCCGTGT GGAACCTGGC TTCTGGTGTG TTCTGGTGTG CCGGAGGCTG CGGAGGCTC
GCACCTCAC CTCCTCGTAC CGTGGGGCT CTTGGTGTG TTCTGGTGTG TTCTGGTGTG TTCTGGTGTG TTCTGGTGTG AGGAGATGTC GTGGAGTGG
406 V E W E S N G Q P E N N Y K T T P P V L D S D G S F F L Y S K L T

2901 GTGGACAGA GCAGGTGGCA GCAGGGGAAC GCTCTCTCAT GCTCCGTGAT GCATGAGGT CTGGACAAAC ACTACACGCA GAGAGGCTC TCCCTGTC
CACCTGTCT CGTCCACCGT CGTCCCCTTG CAGAGAGTA CGAGGCACTA CGGGGCACTA CGTACTCCG GACGTGTGG TGATGTGGT CTCTGGAG AGGGACAG
439 V D K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L S P

3001 CGGGTAATA AGCATGCGAC GGCCCTAGAG TCCCTAACGC TCGGTGCGC CGGGGCGTT TTATTTGTA ACTCATGTGT GACAGCTT CATGATAAG
GCCCAATTAT TCGTAACTG CCGGGATCTC AGGGATTGCG AGCCAAAGGC GGGCCGAAA AAATAACAT TGAGTACAAA CTGTGAAATA GTAGCTATT
473 G K O

3101 CTTTAATGCG GTAGTGTATAC AGCTTTAAAT TCGTAACTGCA GTCAAGGCC GTCAGGCC GTCAGGCC GTCAGGCC GTCAGGCC GTCAGGCC
GAAATTACGC CATCAAATAG TGTCAATTAA AGGATTGCGT CAGTCGGT GACATACATT AGGATTGCG TGGAGTGGC GTCAGTGGG TGGAGTGG

FIG. 20D

3201 TGGATGCTGT AGGCATAGGC TTGGTTATGC CGGTACTGCC CGGGCCTTGG CGGGATATCG TCCATTCCGA CAGCATCGCC AGTCACATAG GCGTGTGCT
ACCTACGACA TCCGTATCCG AACCAAATACG GCGATGACGG CCCGGAGAAC GCCCCTATAGC AGGTAAGGET GTCGTAGGG TCGTGTATAC CGCAAGACGA

FIG. 21A

Inventor: SIMMONS
Docket No.: 11669.120USU1
Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING
YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE
Attorney Name: KATHERINE M. KOWALCHYK
Phone No.: 612.371.5311
Sheet 39 of 50

1 GAATTCAACT TCTCCATACT TTGGATAAGG AAATACAGAC ATGAAAAATC TCATTGCTGA GTTGTATT AAGCTTGCCC AAAAGAAGA AGAGTCGAAT
CTTAAGTGA AGAGGTATGA AACTTATCC TTATATGCTG TACTTTAG AGTAAGACT CAACAATAA TTGAAACGGG TTTCCTCTCT TCTAGCTTA
101 GAACTCTGT CGCAGGTAGA AGCTTGGG ATTATCGTCA CTGCAATGCT TCGAAATATG GCGCAAATG ACCAACAGGG GTGATGAT CAGGTAGAGG
CTTGACAC GCGTCATCT CGGAAACCTC TAATAGCGT GACGTAGA AGCGTATAC CGGGTTAAC CGGTATAC TGGTGTGCG CAACTAACTA GTCCATCTCC
201 GGGGCTTA CGAGGTAAG CCCGATGCCA GCATTCCTGA CGACGATACG GAGCTGTGC GCGATTAGCT AAAGAAAGTA TGAAGACAT CTCGTCAGTA
CCCGGACAT GCTCCATTG GGGCTACGT CGTAAGGACT GCTGCTATGC CTCGACGAC CGCTAATGCA TTTCTTCAT AACCTGTAG GAGCAGTCAT
301 AAAAGTTAT CTTTCAACA GCTGTATCAA AGTGTGTCACG GCCGAGACTT ATAGTCGCTT TTGTTTATT TTGTAACTA GTACGCAAGT
TTTCAATT AAAAGTTCTA GAATTATGAA GAAGAATATC GCATTCTTC TTGCACTAT GTTCGTTTT TCTATTGCTA CAAACGGTA CGCTGATATC
401 TCACGTAAA AGGGTATCTA GAATTATGAA GAAGAATATC GCATTCTTC TTGCACTAT GTTCGTTTT TCTATTGCTA CAAACGGTA CGCTGATATC
AGTGATTT TCCCATAGAT CTAACTCTT CTCTCTATAG CGTAAAGAAG AACGTAGATA CAAGCAAAA AGATAACGAT GTTGGCAT GCGACTATAG
1 M K N I A F L L A S M F V F S I A T N A Y A D I
^STII signal TIR-1
501 CAGCTGACCC AGTCCCCGAG CTCCTGTGCG GCGATAGGGT CACCATGACCG TGCCGTGCGA GTCAAGGGT CGATTAGCAT GGAGTAGCT
GTGACTGG TCAGGGGTC GAGGACAGG CGAGACAC CGTATCCCA GTGGTATGG AGCGACGGT CAGTCCTGCA GCTATAGCTA CCACATATG
26 Q L T Q S P S L S A S V G D R V T I T C R A S Q S V D Y L D G D S Y
601 ACATGAACTG GTATCAACAG AAACCCAGAA AGCTCCGA ACTACTGATT TAGCTGGCCT CGTACCTGGA GTCTGGAGTC CCTTCTGCT TCTCTGGATC
TGTACTGAC CATACTGTC TTGGTCCCT TTGAGGCTT TGATGACTAA ATGGCCGGAA GATGGACCT CAGACCTAG GAAAGAGCGA AGAACCTAG
60 M N W Y Q K P G K A P K L L I Y A A S Y L E S G V P S R F S G S
701 CGGTCTGG ACGGATTCA CTCGACCAT CACAGCTG CAGCGGAAG ACTTCGAAC TTAACTGT CAGCAAAGTC ACCAGGATCC GTACACATT
GCCAAGACCC TGCCTAAAGT GAGACTGGTA GTGTCAGAC GTGCGCTTC TGAAGCTG TAAATGACA GTGTTTCAG TGCTCTAGG CATGTGTA
93 G S G T D F T L T I S S L Q P E D F A T Y Y C Q Q S H E D P Y T F
801 GGACAGGGTA CCAAGGGAGA GATCAAACGA ACTGTGGCTG CACCATCTG CTTCATCTC CGGCCATCTG ATGAGCAGT GAAATCTGGA ACTGCTCTG
CCTGTCCTAT GTTCCACCT CTAGTTGCT TGACACCGAC GTGCTAGACA GAAGTAGACA TACTCGTCAA CTTAGACCT TGACGGAGAC
126 G Q G T K V E I K R T V A A P S V F I F P P S D E Q L K S G T A S V
901 TTGTGTGCT GCTGAATAAC TTCTATCCCA GAGAGGCCA AGTACAGTGG AAGGTGGATA AGCCCTCCA ATCGGGTAAC TCCCAGGAGT TAGCCCATG AGGGTCCT
AACACAGGG CGACTATATG AAGATAGGGT CTCTCCGGTT TCATGTCAC TCCACCTAT TGCGGGAGGT TGCAGTGTAGT CACAGTGTCT
160 V C L N N F Y P R E A K V W K V D N A L Q S G N S Q E S V T E

FIG. 21B

Inventor: SIMMONS
Docket No.: 11669.120USU1
Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING
YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE
Attorney Name: KATHERINE M. KOWALCHYK
Phone No.: 612.371.5311
Sheet 40 of 50

1001 GCAGGACAGC AAGGACAGCA CCTACAGCCT CAGGAGCACC CTGACGCTGA GCAAGCAGA CTAAGGAAA CACAAGCT ACGCCTGCGA AGTCACCCAT
CGTCCTCTCG TTCCCTCTCG GGATCTCGGA STCGTCCTGG GACTGCACT CGTCCTCTGG GATGCTCTT GTGTTTCAGA TGCGGAGCGCT TCACTGGTA
193 Q D S K D S T Y S L S S T L T L S K A D Y E K H K V Y A C E V T H

1101 CAGGGCTGA GCTGCCGCT CACAAAGAGC TTCAAAAGAGC GAGAGTGTAA ATTAAATCCT CTACGCCGA CGCATCGTGG CGAGCTCGGT ACCCGGGAT
GTCCCGCACT CGAGGGGCA GTGGTCTCG AAGTGTCCC CTCTCACAAAT TAATTAGGA GATGCGGCCG GCGTAGGCCA TGCGGCCCTA
226 Q G L S S P V T K S F N R G E C O

1201 CTAGGCCCTAA CGCTCGGTG CGGCCGGGGC TTGCGGAAGC GCATCTCGAA TGAACCTGTT GCGCAGGTAG AAGCTTGGG GATPATCGTC
GATCCGGATT GCGAGGCCAAC GGCGGCCCGC AAAAATAAC AACGGCTGCG CGTAGGCTT ACTTGACACA CGCGTCCATC TICGAAACCT CTATAGCAC
ACTGCAATGC TTGCAATAAT GGCGAAAT GACCAACAGC GGTGATGA TCAGGTAGAG GGGCGCTGT AGAGGTAA GCCCGATGCC AGGATTCTG
TGACGTTAGC AAGCGTTATA CGCGTTATA CTGGTGTG CCAACTAAT AGTCATCTC CGCGGCA TGCTCCATT CGGCGTACGG TCGTAAGAC
1401 ACCGACATAC GGAGCTGCTG CGCGATTAAGC TAAGGAGT ATTGAGAGCAT CCTCGCTAGT AAAAAGTTAA TCTTTTCAAC AGCTGTATA AGCTGTCAC
TGCTGCTATG CCTCGAGAC GCGTAATGC ATTCTCTCAA TAACTCTCAA TTGTTCAATT AGAAAAGTGT CGACAGTAT TTGACAGTGT
1501 GGCCGAGACT TATAGTCGCT TTGTTTTATGT TTGTTAACT AGTAGCGAAG TTACCGTAA AAGGGTATCT AGAATTATGA AGAGAAAT
CGGGCTGGA ATATAGCGGA AACAAAAATAA AAACATGA TAACATGA TCAATGCTTC AATGCAATT TTCCCATAGA TCTTAATACT TCTTCTATA
1

1601 CGCATTCCTT CTGCTCATCTA TGTCTGTTT TTCTATTGCT ACAAACGGT ACGCTCAGGT TCAGCTGCAA GAGTCTGGCC CGGGCTGGT GAAACCATCT
GCGTAAGAA GAACGTAGAT ACAAGAAA AGATAACGA TGTTTGCGCA TGCGAGCTCA AGTCGACGTT CTAGACCGG GCCGGACCA CTTTGCTGAGA
6 A F L L A S M F V F S I A T N A Y A V Q L Q E S G P G L V K P S

73 A S I T Y D G S T N Y N P S V K G R I T I S R D D S K N T F Y L Q

1701 CAGACTCTCT CTTTGACTTG TACTGTTCT GGCTACTCCA TCACCTCGG ATATGCTGG AACTGGATCC GTAGGGCCCC GGTAAAGGGC CTGGATGGG
GTCCTGAGAGA GGAACGTGAC ATGACAAAGA CGATGAGGT AGTGGAGGC TATATGCC TTGACCTAGG CAGTCGGGG CCCATTCCCG GACCTTACCC
39 Q T L S L T C T V S G Y S I T S G Y S W N W I R Q A P G K G L E W V

1801 TTGCACTCAT TAGTATGAC GGATCGACTA ACTATAACC TAGCTCAAG GGCGGATICA CTATAAGTCT CGACGACTCC AAAAACAT TCTACCTGCA
AACGTAGCTA ATGCACTACTG CCTAGCTGTAT TGATATTGGG ATCGAGATT CCGGAGATGT GATATTAGC GCTGCTGAGG TTGTTGTGA AGATGAGCT
73 A S I T Y D G S T N Y N P S V K G R I T I S R D D S K N T F Y L Q

1901 GATGAAACAGC CTGCTGCTG AGGACACTGC CGTCTATTAT TGTCGCTGAG GCAGGCCACTA TTGCGGTCACT CGGCACCTTCG CCCTGGGGG TCAGGAAACC
CTACTCTCTG GACGCACTGAC TCCCTGTGAGC GCAGATAATA ACACGGACTC CGTCTGCTGAGT AAAGCCAGTG ACCGTGAAGC GGACACCCCG AGTTCTGG
106 M N S L R A E D T A V Y Y C A R G S H Y F G H W F A V W G Q G T

2001 CTGGTCAAGC TCTCCCTCGG CTCACCAAG GGCCCATCGG TCTTCCCTGC GGCAACCCCTC TCCAGAGCA CCTCTGGGGG CACAGGGGCC CTGGCTGCC
GACCACTGGC AGAGGAGCCG GAGGTGGTTC CGGGTAGC AGAAGGGGA CGGAGACCCC GTGTCGCCG GACCCGACGG
139 L V T V S S A S T K G P S V F P L A P S S K S T S G G T A A L G C L

1 ^STII Signal TIR-1
1 ^Heavy Chain FRI-SubgroupII consensus sequence

FIG. 21C

Inventor: SIMMONS
Docket No.: 11669.120USU1
Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING
YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE
Attorney Name: KATHERINE M. KOWALCHYK
Phone No.: 612.371.5311
Sheet 41 of 50

2101	GGTCAAGGA	CTACTCCCC	GAACCGGTGAA	CGGTGTCGTG	GAACCTAGGC	GCCCTGACCA	GGGGCGTGGCA	CACCTTCCCG	GCTGTCCCTAC	AGTCCTCTCAGG
173	ACCACTTCT	GATGAAGGG	CTTGGCCACT	GCCACAGCAC	CTTGAGTCCG	CGGACCTGGT	GCCGCACTGGT	GTCAGGAGGC	CGACAGGATG	TGAGGAGTCCAC
2201	ACTCTACTCC	CTCAGCAGGC	TGGTACTGT	GCCCTCTAGC	AGCTTGGCA	CCCGACCTA	CATCTGCAAC	GTGAAATACA	AGCCGCGCA	CACCAAGGTG
206	TGAGATGAGG	GAGTCGTCG	ACCACTGACA	CGGGAGATCG	TGAAACCGT	GGGTCTGGAT	GTAGACGTTG	CACTTAGTGT	TGGGTGCGTT	GTTGTTCCAC
2301	GACAAAGAAG	TTCAGGCCAA	ATCTCTGAC	AAAGACTACA	CATGCCACCC	GTGCGAGCA	CCTGAGCA	TGCGGGAC	GTCAGGTCTC	CTCTTCCCCC
239	CTGTTCTTC	AACTCGGGTT	TAGAACACTG	TTTGAACACTG	GTACGGTGG	CACTGGTGTG	GGACTTGAGG	ACCCCGCTGG	CAGTCAGAAG	GAGAAGGGGG
273	K P K D T L M I S R T P E V T C V V D V S H E D P E L L G G P S V F L F P P									
2401	CAAAACCCAA	GGACACCCCTC	ATGATCTCCC	GGACCCCTGA	GGTCACATGC	GTGGCTGGGG	ACGTGAGGCCA	CGAAGACCT	GAGTCAGGT	TCAACTGTTA
306	GTTTGGGGT	CCTGGGGAG	TACTAGAGGG	CTCTGGGACT	CCAGGTGACG	CACCAACCAC	TGCACTCGGT	GCTTCTGGGA	CTTCAGCTTC	AGTTGACCAT
273	K P K D T L M I S R T P E V T C V V D V S H E D P E L L G G P S V F L F P P									
2501	CGTGGACGGC	GTGGGGTGC	ATATAGCCAA	GACAAAGGCC	CGGGAGGAGC	AGTACACAG	CACTGACCGT	GTGGTCAGCG	TCCTCACCGT	CTCTGACCAAG
306	GCACCTGGCG	CACTCTCACG	TATTACGGTT	CTGTTTGGC	GCCCTCTCTCG	TCACTGGTC	GTGGCATGGCA	CACCACTGGCA	AGGAGTGGCA	GGACGTGGTC
2601	GACTGGTGA	ATGGCAAGGA	GTACAAGTGC	AAGGTCTCCA	ACAAAGCCCT	CCCAAGCCCC	ATCGAGAAMA	CCATCTCAA	AGCCAAAGGG	CAGCCCCGAG
339	CTGACCCACT	TACCGTCTCT	CACTGTCACG	TTCCAGAGGT	TTTTCAGGGG	GGGTGGGG	TAAGCTCTTT	GGTAGAGGGT	TGCTTCTTCCC	GTGGGGGTCTC
2701	AACCAACGGT	GTACACCCCTG	CCCCCATCCC	GGGAAGAGAT	GACCAAGAAC	CAAGCTCAGCC	TGACCTGGCT	GGTCAAGGG	TCTCTATCCA	GGGACATCGC
373	TTGGTGTCCA	CATGGGGAC	GGGGTAGGG	CCCTTCTTA	CTGGTTCTTG	GTCACTGGGG	ACTGGACGGA	CCAGTTTCCG	AAAGTAGGGT	CGCTGTAGGG
2801	CGTGGAGTGG	GAGGCAATG	GGCAGGCCGA	GAACAACTAC	AAGACCAAGC	CTCCCGTGCT	GGACTCCGAC	GGCTCTCTCT	TCCTCTACAG	CAAGCTCACC
406	GCACCTCTACC	CTCTCGTTAC	CCGGCGGCC	CTTGTGATG	TTCTGGTGG	GGGGCACGA	CCTGAGGCTG	CCGAGGAAGA	AGGAGATGTC	GTTCGAGTGG
2901	GTGGCACAGA	GCAGGGAAAC	GCAAGGAAAC	GTCCTCTCAT	GCTCGTGTAT	GCATGAGGCT	CTGCAACACC	ACTACACCA	GAAGAGCCTC	TCCCTCTCTC
439	CACCTGTCT	CGTCCACCGT	CGTCCACCGT	CAGAAGCTA	CGGACACTA	CGTACTCGA	GACTGTGTTGG	TGATGTGGT	CTTCTGGAG	AGGGACAGAG
3001	CGGGTAAATA	AGCATGCGAC	GGCCCTAGAG	TCCCTTAACGC	TGGGTTGCCG	CCGGGGTT	TTTATGTTA	ACTCATGTT	GACAGCTTAT	CATCGATAAG
473	GCCCAATTAT	TGTAACGCTG	CCGGGATCTC	AGGGATTCG	AGCCAAACGGC	GGCCCGCAA	AAATAACAAAT	TGAGTCAA	CTGTGAAATA	GTAGCTTATTC

FIG. 21D

3201 TGGATGCTGT AGGCATAGGC TTGGTTATGC CGGTACTGCC GGGCCTCTTG CGGGATATCG TCCATTCGGA CAGGCATGCC AGTCACATAG GCGTCTGCT
ACCTACGACA TCCGTATCCG AACCAATACTG GGCATGACGG GCCCCATAGC AGGTAAGGCT GTCGTAGGG TCAGTGATAC CGCAGACGA

FIG. 22A

Inventor: SIMMONS
Docket No.: 11669.120USU1
Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING
YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE
Attorney Name: KATHERINE M. KOWALCHYK
Phone No.: 612.371.5311
Sheet 43 of 50

1 GAATTCAACT TCTCCCATACT TTGGATAGG AAATACAGAC ATGAAAATC TCATTGCTGA GTTGTATTTC AAGCTTGCCC AAAAGGAGA AGAGTCGAAT
CTTAAAGTTGA AGGGTATGA AACCTATTC TTTATGCTG TACTTTTAG AGTAACGACT CAAACAATAA TTTCGAXACGG TTTTTCCTCT TCTCAGCTTA
101 GAACTGTGTG CGCAAGCTGAG AGCTTGTGAG ATTATCGTCA CGTCAATGCT TCGCAATGCT TCGCAATATG GCGCAAAATG ACCAACAGGG GTTGATGAT CAGGTAGAGG
CTTGACACAC CGGTCCATCT TCGAAACCTC TAATAGCAGT GACGTTAGA AGCGTTATAC CGCGTTTAC TGGTGTGCG CAACTAATA GTCCATCTCC
201 GGGGCTGTGA CGGGTAAG CCCGATGCCA GATTCCTGA CGACGATAGG GAGCTCTGA CGGATTAGT AAAGAAAGTTA TTGAAGCATC CTCGTCAGTA
CCCGGCACT GCTCCATTTC GGCTTACCGT CGTAAGGACT GCTGCTATGC CTCGACGACG CGCTAATGCA TTTCCTCAAT AACTCGTTAG GAGCAGTCAT
301 AAAAGTTAAAT CTTTCACAA GCTGTCATAA AGTTGTCAAG CGCGAGACCTT ATAGTCGCTT TGTTTTATT TTAAATGTA TTGGTAACTA GTACGCAAGT
TTTCAATTA GAAAGCTGT CGACAGTATT TCAACAGTGC CGGCTCTGAA TATAGCGAA ACAAAATAA AAACATGAT CATGGTTCA
401 TCACGTTAAAGGGTACTA GAATTATGAA GAGAAATATC GCATTCTTC TTGCACTCT TGTGTGTTT TCTATGCTA CAAACGGTA CGCTGATATC
AGTGATTTT TCCCATAGAT CTTAAACTCTTCTCTTATAG CGTAAGAAGG AACGTAGATA CAAAGAAAAA AGATAACGAT GTTGGCCAT GCGACTATAG
1 M K K N I A F L L A S M F V F S I A T N A Y A D I
^STII signal TIR -1 anti-VEGF light chain (1st generation)

501 CAGATGACCC AGTCCCCGAG CTCCTGTCC GGCCTCTGTGG GCGATAGGG CACCATCACC TGCAAGCCAA GTCAAGGATAT TAGCAACTAT TTAACATGGT
GTCCTACTGGG TCAGGGGGTC GAGGGACAGG CGGAGACACC CGCTATCCCA GTGGTAGTGG ACGTGCGTT CAGTCCTATA ATCGTTGATA AATTGACCA
26 Q M T Q S P S L S A S V G D R V T I T C S A S Q D I S N Y L N W Y
60 Q Q K P G K A P K V L I Y F T S S L H S G V P S R F S G S G T
701 ATCAAAGAA ACCAGGAAA GCTTCGAAAG TACTGATTA CTTCACCTCC TCTCTCCACT CTGGAGTCCC TTCTCGCTTC TCTGGATTCGG GTTCCTGGGAC
TAGTGTGTTT TGGTCTCTT CGAGGGCTTC ATGACTTAAT GAAGTGGGG AGRAAGGTGA GACCTCTAGGG AAGAGCGAAG AGACCTAGGC CAAGACCCCTG
93 D F T L T I S S L Q P E D F A T Y Y C Q Q Y S T V P W T F G Q G T
801 AAGGTGGAGA TCAAAGGAC TGTGGCTGCA GCCAGAAAGAC TTGCAACTT ATTACTGTC ACAGTATGTC ACCTGTGCGT GGACGTGTTG ACAGGGTACCC
CTTAAGTGA GACTGGTAGT CGTCAGACGT CGGTCTTCAGT AAGCCTGTTGA TAATGACAGT TGTCTATATCG TGGCACGGCA CCTGCAAACC TGTCCTATGG
126 K V E I K R T V A A P S V F I F P P S D E Q L K S G T A S V V C L L
901 TGAATAACTT CTATCCAGA GAGGCCAAG TAGCTGGAA GGTCGATAC GCCCTCCATT CGGGTAACCT CCAGGAGAGT GTCACAGAGC AGGACAGCAA
TTCCACCTCT AGTTGCTTG ACACCGACGT GGTAGACAGA ACTAGAAGGG CGGTAGACTA CTCGTCAACT TTAGACCTTG ACGAAGACAA CACACGGAGC
160 N N F Y P R E A K V Q W K V D N A L Q S G N S Q E S V T E Q D S K

FIG. 22B

Inventor: SIMMONS
Docket No.: 11669.120USU1
Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING
YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE
Attorney Name: KATHERINE M. KOWALCHYK
Phone No.: 612.371.5311
Sheet 44 of 50

1001 GGACAGGACC TACAGCCCTCA GCAGCACCCCT GACGGCTGAGC AAAGGAGGACT ACGGAAACA CAAAGCTTAC GCCTGCGAAG TCAACCATCA GGGCTTGAGC
CCTGTGCTGG ATGTCGGAGT CGTGTGGAA CTGGGACTCG TTGCTGTGAG TGCTCTTGT GTTCTAGATG CGACGCTC AGTGGTAGT CCCGGACTCG
193 D S T Y S L S T L T L S K A D Y E K H K V Y A C E V T H Q G L S

1101 TCGCCCCCTCA CAAAGAGCTT CAAACAGGGAA GAGTGTAAAT TAAATCCTCT ACGCCGGACG CATCGTGGGG AGCTCGGTAC CGGGGGATCT AGGGCTAACG
AGCGGGCACT GTTTCCTGAA GTTGTCCCCT CTCACAAATTAA ATTAGGAGA TGCGGCCCTGC GTAGCACCGC TCGAGCCATG GCCCCCTAGA TCCGGATTGC
226 S P V T K S F N R G E C O

1201 CTCGGTTGCC GCGCGGGT TTATTGTT GCGGACGCGC ATCTGAAATG AACTGTTGTC GCAGGTAGAA GCTTTGGAGA TTATGTCAC TGCAATGCTT
GAGGCCAACGG CGGGCCGGAA AAAATAACAA CGGGCTGCGG TAGAGCTTAC CGGCCATCTT CGAAACCTCT AATAGCACTG ACGTGAGAA

1301 CGCAATATGG CGCAAAATGA CCAACAGGGG TTGATTGATC AGGTAGAGGG GGGGGTGTAC GAGGTAAAGC CCGATGGCCAG CATTCCGTAC GACGGATAACGG
GGGTATATCC CGGTGTTACT GGTGTTGCGCC FACAATCTAG TCCATCTCCC CCGGGACATG CTCCATTTGC GGCTACGGTC GAAAGGACTG CTGCTATGCC

1401 AGCTGCTGG CGATTACGTA AAGAAGTTAT TGAAGGATC TCGTGTAGTTAA AAAGGTAATC TTTCACAG CGTGTACAAA CGTGTACCGG CGAGGACTTA
TCGACGGCC GCTAAATGCACT TTCTTCAATA ACTCTGAGG AGCACTCAATT TTCAATTAG AAAAGTGTCA GACAGTATT CAACAGTGCC GGCTCTGAAT

1501 TAGTCGCTT GTTTTATT TTAATGTT TTGTAATCTAG TAGCGTAAAGTT CACGTAAAGA GGTATCTAG AATTATGAAAG AAGAATATCG CATTCTCT
ATCAGGAAAA CAAATAAAA AAATTACATA AACATTGATC ATGGCTCAA GTGCAATTTT CCATAGATC TPAATACITC TCTCTTATGC GTAAAGAAGA
1 M K K N I A F L L

1601 TGCATCTATG TTGTTTTCTT CTATTGCTAC AACGCGTAC GCTGAGGGTT AGCTGGTGA GTCTGGCTGT GGCTGGTGC AGCCAGGGGG CTCTACTCGT
ACGTAGATAC AAGCAAAAA GATAACGATG TTGCGCATG CGACTCAAG TCGACCACT CAGACCGCCA CGGACCCAG TCGGTCCCCC GAGTGAGGCA
10 A S M F V F S I A T N A Y A E V Q L V E S G G G L V Q P G G S L R
^ anti-VEGF (1st generation) heavy chain

1701 TTGTCCTG CAGCTCTGG CTATACCTTC ACCAACTATG GTATGAACTG GGTGGACTG GGTCCGTCAG GCCCCGGGTAA AGGGCTGGAA ATGGTTGGA TGGATTAAACA
AACAGGACAC GTGGAAGACC GATATGGAA TGTTGATAC CATACTTGAC CGGGGACCT TCCGGACCT ACCPAATTG
43 L S C A A S G Y T F T N Y G M N W V R Q A P G K G L E W V G W I N T

1801 CCTATACGG TGAACCGACC TATGGCTGGG ATTCAAAAGC TCGTTCAACT TTGAGCTTAG ACACCTCCA GTGACAGCA TACCTGAGA TGAACAGCCT
GGATATGGC ACTTGGCTGG ATAGCACGCC TAAAGTTGC AGCAAAAGTGAA AAGTCGAATC TGTTGAGETT CAGCTGTCGT ATGGAGTCT ACTGTCGGA
77 Y T G E P T Y A A D F K R R F T F S L D T S K S T A Y L Q M N S L

1901 GCGTGTAG GACACTGCGC TCTATTACTG TGCAAAGTAC CCCCACTATT ATGGAGGAG CCACITGGTAT TTGAGCTGTCT GGGGTCAAGG AACCTGGTC
CGCACGACTC CTGTGACGCC AGATAATGAC AGTTTCATG GGGGTGATAA TACCTCGTC GTGACCAAA AAGTCGAGA CCCAGTCTCC TTGGACCAAG
110 R A E D T A V Y Y C A K Y P H Y Y G S S H W Y F D V W G Q G T L V

2001 ACCGTCCTCT CGGCCCTCCAC CAAGGGCCCA TCGGTCTTC CCGCTGGCAAC CTCCCTCAAG AGCACCTCTG GGGGCACAGC GGCCCTGGGC TGCCTGGTCA
TGGCAGGAGCA GCGGGAGGTG GTTCCGGGT AGCCAGAAGG GGGACCGTGG TCGTGGAGAC CCCAGTCTCC CGGGACCCG ACGGACAGT
143 T V S S A S T K G P S V F P L A P S S K S T S G G T A A L G C L V K

^STII signal TIR-1

FIG. 22C

Inventor: SIMMONS
Docket No.: 11669.120USU1
Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING
YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE
Attorney Name: KATHERINE M. KOWALCHYK
Phone No.: 612.371.5311
Sheet 45 of 50

2101 AGGACTACTT CCCCGAACCC GTGACCGCTGT CGTGGAACTC ACCAGGCCCTG TGACACCCCTT CCGGGCTGTCTA CTCAGGCCCT CAGGACTCTA
177 TCCCTGATGAA GGGGGTGGG CACTGCCACA GCACCTTGGAC TCCGGGGGAC ACGTGGCGC GGGCGACAG GATGTCAGGA GTCCCTGAGAT
177 D Y F P E P V T V S W N S G A L T S G V H T F P A V L Q S S G L Y
2201 CTCCTCTAACC AGCGTGTGTA CTGTGCCCTC TAGCAGCTTG GGCACCCAGA CCTACATCTG CAACGTGAAT CACAAGCCCA GCAACACCAA GTGGACAAG
210 S L S S V V T V P S S L G T Q T Y I C N V N H K P S N T K V D K
2301 AAAGTGTGAC CCAAATCTCTG TGACAAAATC CACACATGCC CACCGTGGCC AGCACCTGAA CTCCCTGGGG GACCGTCACT GTGTCGGGT CGTGGCTGTT CCACCTGTT
243 K V E P K S C D K T H T C P P C P A P E L L G G P S V F L F P P K P
2401 CCAAGGACAC CCTCTATGATC TCCCGGACCC CTCAGGTCA ATGCGTGGTG GTGGACGTGA GGCACGAAGA CCCTGAGGT AAGTCAACT GGTAGTGGAA
277 K D T L M I S R T P E V T C V V V D V S H E D P E V K F N W Y V D
277 GGTTCCTGTS GGAGTACTAG AGGGCTGGG GACTCCAGTG TACCCACAC CACCTGACT CGGTGCTTCT GGGACTTCAG TTCAAGTTGA CCATGACCT
2501 CGGGTGTGAG GTGCAATAATG CCAAGACAAA GCGGGGGAG GAGGAGTACA ACAGGACCTA CGGTGTGGTC AGGTCTCTCA CGGTCTGCA CCAGGACTGG
270 G V E V H N A K T K P R E E Q Y N S T Y R V V S V L T V L H Q D W
270 GCGCACCTC CAGTATTAC GGTCTGTT CCGCGCCCTC CTCGTCATGT TGTGTCAT GTGTCGAT GGACACCAAG TCGAGGAAT GGTCTGACCC
310 V Y T L P P S R E E M T K N Q V S L T C L V K G F Y P S D I A V E
310 CTGAATGCA AGGAGTACAA GTGCAAGGT TCCAACAAAG CCCTCCAGC CCCCATCGAG AAAACCATCT CCAAAGCCAA AGGGCAGCCC CGAGAACCC
343 L N G K E Y K C K V S N K A L P A P I E K T I S K A K G Q P R E P Q
343 GACTTACCT TCCTCATGTT CAGTTCAGG AGGTGGTTTC GGGTAGCTC TTGGTGTAGA GGTTTCGGTT TCCCGTGGG GCTCTGGG
2701 AGGTGTACAC CCTGCCCTCA TCCGGGAAG AGATGACCAA GAACCAAGTC AGCCCTGACT GCCTGGTCAA AGGCTTCTAT CCCAGGACA TCGGGTGGAA
377 TCCACATGTS GGACGGGGGGT AGGGCCCTC TCTACTGGT CTCGGTCA CGGGACTCGA CGGACCAATT TCCGAAGATA GGTOGCTGT AGGGCACCT
377 V Y T L P P S R E E M T K N Q V S L T C L V K G F Y P S D I A V E
2801 GTGGGAGGC AATGGGCAGC CGGAGAACAA CTACAAGAC CACGCCCTCC TGCTGGACTC CGACGGCTCC TTCTTCCTCT ACAGCAAGCT CACCGTGGAC
410 CACCCCTCTG TTACCGTGTG GCCTCTGTG GATGTCCTGG TGCGGGGG ACGACCTGAG GCTGCCGAGG AAGAAGGAGA TGTGTCAGA GTGGCAGCTG
410 W E S N G Q P E N N Y K T T P P V L D S D G S F F L Y S K L T V D
2901 AAGAGGAGGT GGCAAGGG GAAAGTGTCTTC TCAAGTGTCCG TGATGCTATGA GGACTGAC ACCACTACA CGCAGAAGAG CCTCTCCCTG TCTCCGGTA
443 K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L S P G K
443 TTCTCTCTCA CGGTGTCTCC CTTGCAAGAG AGTAGGAGGC ACTAGTACT CCGAGACGT TTGGTGTATGT GGTCTTCTC GGAGAGGGAC AGAGGCCCAT
477 O
3001 AATAAGGTG CGACGGCCCT AGAGTCCCTA ACCTCGGT GCCGGGGC GTTTTTATT GTAACTCAT GTTGACAGC TTATCATCGA TAAGCTTAA
3001 TTATTCGTAC GCTGCCGGAA TCTCAGGGAT TGCGAGCCAA CGGCCGGCG CAAAAAATTA CATTGAGTA CAAACTGTG AAATAGTAGCT ATTGAAATT
3101 TGCGGTGTT TATCAGTGT AAAATTGCTAA CGGAGTCAGG CACCGTGTAT GAAATCTAAC AATGCGCTCA TCGTCATCT CGGCACCGTC ACCCTGGATG
3101 ACGCCCATCAA ATAGTGTCAA TTAACTGATT CGGTCACTC GTCAGTGTG TTACGAGAT AGCAAGTGA GCGTGGCAG TGGGACCTAC
3201 CTGTAGGGAT AGGTGGT ATGCGGTAC TCGGGGCT CTCGGGGAT ATCGTCCATT CGACAGCAT CGCCAGTCA TATGGCTGTC GCGTGGCT
3201 GACATCCGTA TCCGAACCA TAGGCCATG AGGGCCCTA TAGCAGGTA GGCTGTGTA GCGTGGCAGC AGCATGGCGA

FIG. 23A

29nt 1'-VEGF (VNTR version) 1981 Expression Cassette with heavy chain FR1, 2, 3, 4=consensus subgroup I

FIG. 23B

1301 CCTAATATGG CCAACATGA CCAACGGGG TTTTATTTC AGGTAGAGGG GGGGCTGTAC GAGGTAAAGC CCGATGCCAG CATTCTGAC GACCATACGG
GGGTATACC GCTTTRACT GGTTCATCC AACTAATAG TCCATCTCCC CCGGACATG CTCCATTTCG GGCTACGGTC GAAAGGACTG CTGCTATGCC
ahdi/eam1105I
bsmI
1401 AGCTGCTGGG CGATTACGTA AAGAAGTTAT TGTAGGATCC TCGTCAGTAA AAAGTTAATC TTTCAACAG CTGTCTATAA GTTGTACGG CGAGAGCTTA
TCGACGACGC GCTTATGGAT TTCTTCATA ACTTCGTTAG AGCACTCATT TTTCATTAG AAAAGTTGTC GACAGTATT CAACAGTGGC AGCTCTGAA
eagI
1501 TAGTCGCTT GTTTTATT TTAATGTT TGTAACTAG TACGCAAGTT CACGTAAAA CGGTATCTAG ATTATGAA AGAAATATCG CATTCTCTTC
ATCAGGAAA CAAATAAA AAATTACATA AACATTGATC ATGCTTCAA GTGCTTAA GTGCATTTT CCCATAGATC TAAATACTTC TTCTTATAGC GTAAAGAGA
1
xbaI
1601 TGCATCTATG TTGCTTTT CTATTGCTAC AAACGGTAC GCTCAGGTT AGCTGGTCA AGCTGGTCA GTCCTGGCA GAGGTAAAGA AGCCAGGGC TTGCTGTTAA
ACGTAGATAC AAGCAAAAAA GATAACGATG TTGCGCAGT CGAGTCCAAAG TCGACCAGT CAGACCCGGT CTCCACTTTT TCGCTCCCCG AAGTCATT
10 A S M P V F S I A T N A Y A Q V Q L V Q S G A E V K K P G A S V K
^start xVEGF (VNERK) heavy chain (consensus subgroup I framework
^FR1=Subgroup I
bsmI/splI
1701 GATTCCTTC ACCAACTATG GTATAAATG GGTCGGTCA AGCTGGTCA GTCCTGGCA ATGGATGGGA TGGTAAACA
CATAGGACAT TTGAAAGACC GATAATGAG TGGTGTATAAC CATATTTGAC CCAGGAGTC CGGGCCAG TCCGGACCT TACCTACCT ACCTATTGT
43 V S C K A S G Y T P T N Y G I N W V R Q A P G Q G L E W M G W I N T
^FR2=Subgroup I
alwNI
1801 CCTATACGGG TGAACGACCC TATGCTCGG ATTCAACG TCGCTTACT ATCACTGCTG ACACCTCCAC TAGCACCGA TACATGGAC TGTCTGGCT
GAATATGCC ACTTGCTGG ATACGACGCC TAAGTGTGC AGCACTATGA TAGTGAAGAC TGTGGAGGTG ATGTGTCTG ATGTACCTTG ACATATGGA
77 Y T G B P T Y A D P K R R V T I T A D T S T A Y M E L S S L
^FR3=Subgroup I
apalI/snoI
1901 CGCTCTGG GAGACTCCG TCTTATTCTG CC3CACTATT ATGTGAACGA GC3GAAGAGC CACTCTATT TCGACGTCTG CGCTGAGGA
CGAGAGCTC CTGACGGC AGATTAATGAC AGCTGCAATG GCGCTGATAA TACACTGGT CGCTGCTCG GTGACCTAA AGCTGAGAC CCCAGTCTC
110 R S B D T A V Y Y C A R Y P H Y Y V N E R K S H W Y F D V W G Q G
^FR4= same sequence for subgroup I, II and III

FIG. 23D

xmaI/papAI bsp1407I/bspGI smaI earI/ksp632I sexAI bspMI
 2701 GAGAACCA GGTGTACACC CTCGCCCCAT CCCGGAAAGA GATGACCAAG AACCAAGGTCA GCCTAACCTG CCTGGTCAA GGCTTCTATC CGAGGACAT
 CTCCTGGT CCACATGTTG GACGGGGGTA GGGCCCTCT CTACTGGTC 377 E P Q V Y T L P P S R B M T K N Q V S L T C L V K G F Y P S D I

bspDI 2801 CCCCTGGAG TGGAGAGCA ATGGGAGCC GAGAACAC TACAAGACCA CGCTCCCGT GCTGAACTCC GACGGCTCT TCTTCCTCTA CAGGAAGCTC
 GCGGCAACCTC ACCCTCTCTT TACCTCTCTG CCTCTCTG ATGCTCTG ATGCTCTG 410 A V E W S N G Q P E N N Y K T T P P V L D S D G S F F L Y S K L

bspAI xmaI bspI ppu10I
 2901 ACCCTGACA AGAGGAGG GAGAGGGG AACCTCTCTT CTCGCTCTGT GATGCTGGAG GCTCTCCACA ACCACTAAC GCGAGAAGGG CTCCTCCCTGT
 TGGACCTCT TCTCTCTAC CGCTCTCTC CGAGACCTCT CTACGTAAC 443 T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L S L

bspAI xmaI bspI nsI/avaiII bsgI earI/ksp632I
 3001 CTCCGGTA ATAGGATGC GAGGCCCTAA GAGTCTCTAA CGCTGGTGT CGGCGGGCG TTTTTTATTG TAACTCATG TTGACAGCT TATCATCT
 GAGGCCCAT TATTGGTAGC CTGCGGGAT CTGCGGGAT 477 P G K O

HindIII 3101 AGCTTTAACT GCGTAGTTT ATCACAGTTA AATTGCTAAC GCAAGTCAAC ACCGTGTATG AAATCTAAC ATGGCTCAT CGTCATCCTC GCGACCCGCA
 TTGAAATTAA CCGCCATCAA TAGCTCTCAT TTAACGATT CGTCTGCTG TGGACATAC TTGAGTTG TACGGAGTA GGAGTAGGAG CGTCTGGCTGT

ecORV 3201 CCCTGGATGC TGTAGGCATA GGCTGGTAACT GCGGGCTCTC TTGGGGATA TCGTCCATTG CGACGGATC GCCAGTCACT ATGGCTCTCT
 GGGACCTACG ACATCGTAT CGAACCAAT ACGGCCATGA CGGGGGAG AACGCCCTAT AGGGTTAAG GCTGTCGTAG CGGTCACTGA TACGGACCA
 3301

Inventor: SIMMONS
 Docket No.: 11669.120USU1
 Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING
 YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE
 Attorney Name: KATHERINE M. KOWALCHYK
 Phone No.: 612.371.5311
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FIG. 23E

> length: 3300

aatII (GACGTC):	1983
acc65I (GGTACCC):	795 1176
ageI (ACCCGTT):	1806 2126
ahaIII (TTTAA):	590
ahdI (GACCTTTCGTC):	346 1495 2380
alw26I (CAGGAGCTG):	1089 1770 2359
alw44I (GTCGAC):	1930 2169